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(54) Title: INDUCING CELLULAR IMMUNE RESPONSES TO HER2/neu USING PEPTIDE AND NUCLEIC ACID COMPOSITIONS

(57) Abstract: This invention uses our knoeledge of the mechanisms by which antigen is recognized by T cells to identify and prepare HER2/neu epitopes, and to develop epitope-based vaccines directed towards HERS2/neu-bearing tumors. More specifically, this application communicates our discovery of pharmaceutical compositions and methods of use in the prevention and treatment of cancer.

INDUCING CELLULAR IMMUNE RESPONSES TO HER2/neu USING PEPTIDE AND NUCLEIC ACID COMPOSITIONS

I. BACKGROUND OF THE INVENTION

A growing body of evidence suggests that cytotoxic T lymphocytes (CTL) are important in the immune response to tumor cells. CTL recognize peptide epitopes in the context of HLA class I molecules that are expressed on the surface of almost all nucleated cells. Following intracellular processing of endogenously synthesized tumor antigens, antigen-derived peptide epitopes bind to class I HLA molecules in the endoplasmic reticulum, and the resulting complex is then transported to the cell surface. CTL recognize the peptide-HLA class I complex, which then results in the destruction of the cell bearing the HLA-peptide complex directly by the CTL and/or via the activation of non-destructive mechanisms, *e.g.*, activation of lymphokines such as tumor necrosis factor-α (TNF- α) or interferon-γ (IFNγ) which enhance the immune response and facilitate the destruction of the tumor cell.

Tumor-specific helper T lymphocytes (HTLs) are also known to be important for maintaining effective antitumor immunity. Their role in antitumor immunity has been demonstrated in animal models in which these cells not only serve to provide help for induction of CTL and antibody responses, but also provide effector functions, which are mediated by direct cell contact and also by secretion of lymphokines (*e.g.*, IFNγ and TNF- α).

A fundamental challenge in the development of an efficacious tumor vaccine is immune suppression or tolerance that can occur. There is therefore a need to establish vaccine embodiments that elicit immune responses of sufficient breadth and vigor to prevent progression and/or clear the tumor.

The epitope approach employed in the present invention represents a solution to this challenge, in that it allows the incorporation of various antibody, CTL and HTL epitopes, from discrete regions of a target tumor-associated antigen (TAA), and/or regions of other TAAs, in a single vaccine composition. Such a composition can simultaneously target multiple dominant and subdominant epitopes and thereby be used to achieve effective immunization in a diverse population.

HER2/neu (or erbB-2) is a 185 kD transmembrane protein with tyrosine kinase activity that has a structure similar to the epidermal growth factor receptor (Coussens et al., Science 230:113-119, 1985; Bargmann et al., Nature 319:226-230, 1986; Yamamoto et al., Nature 319:230-234, 1986). Amplification of the Her2/neu gene and/or overexpression of the protein have been reported in many human adenocarcinomas of the breast, ovary, uterus, prostate, stomach, esophagus, pancreas, kidney, and lung (*see, e.g.*, Slamon *et al.*, *Science* 235:177-182, 1987 and *Science* 244:707-712, 1989; Borg *et al.*, *Cancer Res.* 50:4332-4337, 1990; Lukes *et al.*, *Cancer* 73:2380-2385, 1994; Kuhn *et al.*, *J. Urol.* 150:1427-1433, 1993; Sadasivan *et al.*, *J. Urol.* 150:126-131, 1993; Yonemura *et al.*, *Cancer Res.* 51:1034-1038, 1991; Kameda *et al.*, *Cancer Res.* 50:8002-8009, 1990; Houldsworth *et al.*, Cancer Res. 50:6417-6422, 1990; Yamanaka *et*

al., *Human Path.* 24:1127-1134, 1993; Weidner *et al.*, *Cancer Res.* 50:4504-4509, 1990; Kern *et al.*, *Cancer Res.* 50:5184-5187, 1990; and Rachwal *et al.*, *Br. J. Cancer* 72:56-64, 1995). This widespread expression on cancer cells makes HER2/neu an important target for immunotherapy.

The information provided in this section is intended to disclose the presently understood state of the art as of the filing date of the present application. Information is included in this section which was generated subsequent to the priority date of this application. Accordingly, information in this section is not intended, in any way, to delineate the priority date for the invention.

II. SUMMARY OF THE INVENTION

This invention applies our knowledge of the mechanisms by which antigen is recognized by T cells, for example, to develop epitope-based vaccines directed towards TAAs. More specifically, this application communicates our discovery of specific epitope pharmaceutical compositions and methods of use in the prevention and treatment of cancer.

Upon development of appropriate technology, the use of epitope-based vaccines has several advantages over current vaccines, particularly when compared to the use of whole antigens in vaccine compositions. For example, immunosuppressive epitopes that may be present in whole antigens can be avoided with the use of epitope-based vaccines. Such immunosuppressive epitopes may, *e.g.*, correspond to immunodominant epitopes in whole antigens, which may be avoided by selecting peptide epitopes from non-dominant regions (*see, e.g.*, Disis *et al.*, *J. Immunol.* 156:3151-3158, 1996).

An additional advantage of an epitope-based vaccine approach is the ability to combine selected epitopes (CTL and HTL), and further, to modify the composition of the epitopes, achieving, for example, enhanced immunogenicity. Accordingly, the immune response can be modulated, as appropriate, for the target disease. Similar engineering of the response is not possible with traditional approaches.

Another major benefit of epitope-based immune-stimulating vaccines is their safety. The possible pathological side effects caused by infectious agents or whole protein antigens, which might have their own intrinsic biological activity, is eliminated.

An epitope-based vaccine also provides the ability to direct and focus an immune response to multiple selected antigens from the same pathogen (a "pathogen" may be an infectious agent or a tumor-associated molecule). Thus, patient-by-patient variability in the immune response to a particular pathogen may be alleviated by inclusion of epitopes from multiple antigens from the pathogen in a vaccine composition.

Furthermore, an epitope-based anti-tumor vaccine also provides the opportunity to combine epitopes derived from multiple tumor-associated molecules. This capability can therefore address the problem of tumor-to tumor variability that arises when developing a broadly targeted anti-tumor vaccine for a given tumor type and can also reduce the likelihood of tumor escape due to antigen loss. For example, a breast cancer tumor in one patient may express a target TAA that differs from a breast cancer tumor in another patient. Epitopes derived from multiple TAAs can be included in a polyepitopic vaccine that will target both breast cancer tumors.

One of the most formidable obstacles to the development of broadly efficacious epitope-based immunotherapeutics, however, has been the extreme polymorphism of HLA molecules. To date, effective non-genetically biased coverage of a population has been a task of considerable complexity; such coverage has required that epitopes be used that are specific for HLA molecules corresponding to each individual HLA allele. Impractically large numbers of epitopes would therefore have to be used in order to cover ethnically diverse populations. Thus, there has existed a need for peptide epitopes that are bound by multiple HLA antigen molecules for use in epitope-based vaccines. The greater the number of HLA antigen molecules bound, the greater the breadth of population coverage by the vaccine.

Furthermore, as described herein in greater detail, a need has existed to modulate peptide binding properties, *e.g.*, so that peptides that are able to bind to multiple HLA molecules do so with an affinity that

will stimulate an immune response. Identification of epitopes restricted by more than one HLA allele at an affinity that correlates with immunogenicity is important to provide thorough population coverage, and to allow the elicitation of responses of sufficient vigor to prevent or clear an infection in a diverse segment of the population. Such a response can also target a broad array of epitopes. The technology disclosed herein provides for such favored immune responses.

In a preferred embodiment, epitopes for inclusion in vaccine compositions of the invention are selected by a process whereby protein sequences of known antigens are evaluated for the presence of motif or supermotif-bearing epitopes. Peptides corresponding to a motif- or supermotif-bearing epitope are then synthesized and tested for the ability to bind to the HLA molecule that recognizes the selected motif. Those peptides that bind at an intermediate or high affinity *i.e.*, an IC_{50} (or a K_D value) of 500 nM or less for HLA class I molecules or an IC_{50} of 1000 nM or less for HLA class II molecules, are further evaluated for their ability to induce a CTL or HTL response. Immunogenic peptide epitopes are selected for inclusion in vaccine compositions.

Supermotif-bearing peptides may additionally be tested for the ability to bind to multiple alleles within the HLA supertype family. Moreover, peptide epitopes may be analogued to modify binding affinity and/or the ability to bind to multiple alleles within an HLA supertype.

The invention also includes embodiments comprising methods for monitoring or evaluating an immune response to a TAA in a patient having a known HLA-type. Such methods comprise incubating a T lymphocyte sample from the patient with a peptide composition comprising a TAA epitope that has an amino acid sequence described in, for example, Tables XXIII, XXIV, XXV, XXVI, XXVII, and XXXI which binds the product of at least one HLA allele present in the patient, and detecting for the presence of a T lymphocyte that binds to the peptide. A CTL peptide epitope can, for example, be used as a component of a tetrameric complex for this type of analysis.

An alternative modality for defining the peptide epitopes in accordance with the invention is to recite the physical properties, such as length; primary structure; or charge, which are correlated with binding to a particular allele-specific HLA molecule or group of allele-specific HLA molecules. A further modality for defining peptide epitopes is to recite the physical properties of an HLA binding pocket, or properties shared by several allele-specific HLA binding pockets (*e.g.* pocket configuration and charge distribution) and reciting that the peptide epitope fits and binds to the pocket or pockets.

As will be apparent from the discussion below, other methods and embodiments are also contemplated. Further, novel synthetic peptides produced by any of the methods described herein are also part of the invention.

III. BRIEF DESCRIPTION OF THE FIGURES

not applicable

IV. DETAILED DESCRIPTION OF THE INVENTION

The peptide epitopes and corresponding nucleic acid compositions of the present invention are useful for stimulating an immune response to a TAA by stimulating the production of CTL or HTL responses. The peptide epitopes, which are derived directly or indirectly from native TAA protein amino acid sequences, are able to bind to HLA molecules and stimulate an immune response to the TAA. The complete sequence of the TAA proteins to be analyzed can be obtained from GenBank. Peptide epitopes and analogs thereof can also be readily determined from sequence information that may subsequently be discovered for heretofore unknown variants of particular TAAs, as will be clear from the disclosure provided below.

A list of target TAA includes, but is not limited to, the following antigens: MAGE 1, MAGE 2, MAGE 3, MAGE-11, MAGE-A10, BAGE, GAGE, RAGE, MAGE-C1, LAGE-1, CAG-3, DAM, MUC1, MUC2, MUC18, NY-ESO-1, MUM-1, CDK4, BRCA2, NY-LU-1, NY-LU-7, NY-LU-12, CASP8, RAS, KIAA-2-5, SCCs, p53, p73, CEA, Her 2/neu, Melan-A, gp100, tyrosinase, TRP2, gp75/TRP1, kallikrein, PSM, PAP, PSA, PT1-1, B-catenin, PRAME, Telomerase, FAK, cyclin D1 protein, NOEY2, EGF-R, SART-1, CAPB, HPVE7, p15, Folate receptor CDC27, PAGE-1, and PAGE-4.

The peptide epitopes of the invention have been identified in a number of ways, as will be discussed below. Also discussed in greater detail is that analog peptides have been derived and the binding activity for HLA molecules modulated by modifying specific amino acid residues to create peptide analogs exhibiting altered immunogenicity. Further, the present invention provides compositions and combinations of compositions that enable epitope-based vaccines that are capable of interacting with HLA molecules encoded by various genetic alleles to provide broader population coverage than prior vaccines.

IV.A. Definitions

The invention can be better understood with reference to the following definitions, which are listed alphabetically:

A "computer" or "computer system" generally includes: a processor; at least one information storage/retrieval apparatus such as, for example, a hard drive, a disk drive or a tape drive; at least one input apparatus such as, for example, a keyboard, a mouse, a touch screen, or a microphone; and display structure. Additionally, the computer may include a communication channel in communication with a network. Such a computer may include more or less than what is listed above.

A "construct" as used herein generally denotes a composition that does not occur in nature. A construct can be produced by synthetic technologies, *e.g.*, recombinant DNA preparation and expression or chemical synthetic techniques for nucleic or amino acids. A construct can also be produced by the addition or affiliation of one material with another such that the result is not found in nature in that form.

"Cross-reactive binding" indicates that a peptide is bound by more than one HLA molecule; a synonym is degenerate binding.

A "cryptic epitope" elicits a response by immunization with an isolated peptide, but the response is not cross-reactive *in vitro* when intact whole protein which comprises the epitope is used as an antigen.

A "dominant epitope" is an epitope that induces an immune response upon immunization with a whole native antigen (see, *e.g.*, Sercarz, *et al.*, *Annu. Rev. Immunol.* 11:729-766, 1993). Such a response is cross-reactive *in vitro* with an isolated peptide epitope.

With regard to a particular amino acid sequence, an "epitope" is a set of amino acid residues which is involved in recognition by a particular immunoglobulin, or in the context of T cells, those residues necessary for recognition by T cell receptor proteins and/or Major Histocompatibility Complex (MHC) receptors. In an immune system setting, *in vivo* or *in vitro*, an epitope is the collective features of a molecule, such as primary, secondary and tertiary peptide structure, and charge, that together form a site recognized by an immunoglobulin, T cell receptor or HLA molecule. Throughout this disclosure epitope and peptide are often used interchangeably.

It is to be appreciated that protein or peptide molecules that comprise an epitope of the invention as well as additional amino acid(s) are within the bounds of the invention. In certain embodiments, there is a limitation on the length of a peptide of the invention which is not otherwise a construct as defined herein. An embodiment that is length-limited occurs when the protein/peptide comprising an epitope of the invention comprises a region (i.e., a contiguous series of amino acids) having 100% identity with a native sequence. In order to avoid a recited definition of epitope from reading, *e.g.*, on whole natural molecules, the length of any region that has 100% identity with a native peptide sequence is limited. Thus, for a peptide comprising an epitope of the invention and a region with 100% identity with a native peptide sequence (and which is not otherwise a construct), the region with 100% identity to a native sequence generally has a length of: less than or equal to 600 amino acids, often less than or equal to 500 amino acids, often less than or equal to 400 amino acids, often less than or equal to 250 amino acids, often less than or equal to 100 amino acids, often less than or equal to 85 amino acids, often less than or equal to 75 amino acids, often less than or equal to 65 amino acids, and often less than or equal to 50 amino acids. In certain embodiments, an "epitope" of the invention which is not a construct is comprised by a peptide having a region with less than 51 amino acids that has 100% identity to a native peptide sequence, in any increment of (50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5) down to 5 amino acids.

Certain peptide or protein sequences longer than 600 amino acids are within the scope of the invention. Such longer sequences are within the scope of the invention so long as they do not comprise any contiguous sequence of more than 600 amino acids that have 100% identity with a native peptide sequence, or if longer than 600 amino acids, they are a construct. For any peptide that has five contiguous residues or less that correspond to a native sequence, there is no limitation on the maximal length of that peptide in order to fall within the scope of the invention. It is presently preferred that a CTL epitope of the invention be less than 600 residues long in any increment down to eight amino acid residues. "Human Leukocyte Antigen" or "HLA" is a human class I or class II Major Histocompatibility Complex (MHC) protein (*see*, *e.g.*, Stites, *et al.*, IMMUNOLOGY, 8TH ED., Lange Publishing, Los Altos, CA, 1994).

An "HLA supertype or family", as used herein, describes sets of HLA molecules grouped on the basis of shared peptide-binding specificities. HLA class I molecules that share somewhat similar binding

affinity for peptides bearing certain amino acid motifs are grouped into HLA supertypes. The terms HLA superfamily, HLA supertype family, HLA family, and HLA xx-like molecules (where xx denotes a particular HLA type), are synonyms.

Throughout this disclosure, results are expressed in terms of "IC_{50}'s." IC_{50} is the concentration of peptide in a binding assay at which 50% inhibition of binding of a reference peptide is observed. Given the conditions in which the assays are run (*i.e.*, limiting HLA proteins and labeled peptide concentrations), these values approximate K_D values. Assays for determining binding are described in detail, *e.g.*, in PCT publications WO 94/20127 and WO 94/03205. It should be noted that IC_{50} values can change, often dramatically, if the assay conditions are varied, and depending on the particular reagents used (*e.g.*, HLA preparation, *etc.*). For example, excessive concentrations of HLA molecules will increase the apparent measured IC_{50} of a given ligand.

Alternatively, binding is expressed relative to a reference peptide. Although as a particular assay becomes more, or less, sensitive, the IC_{50}'s of the peptides tested may change somewhat, the binding relative to the reference peptide will not significantly change. For example, in an assay run under conditions such that the IC_{50} of the reference peptide increases 10-fold, the IC_{50} values of the test peptides will also shift approximately 10-fold. Therefore, to avoid ambiguities, the assessment of whether a peptide is a good, intermediate, weak, or negative binder is generally based on its IC_{50}, relative to the IC_{50} of a standard peptide.

Binding may also be determined using other assay systems including those using: live cells (*e.g.*, Ceppellini *et al.*, *Nature* 339:392, 1989; Christnick *et al.*, *Nature* 352:67, 1991; Busch *et al.*, *Int. Immunol.* 2:443, 19990; Hill *et al.*, *J. Immunol.* 147:189, 1991; del Guercio *et al.*, *J. Immunol.* 154:685, 1995), cell free systems using detergent lysates (*e.g.*, Cerundolo *et al.*, *J. Immunol.* 21:2069, 1991), immobilized purified MHC (*e.g.*, Hill *et al.*, *J. Immunol.* 152, 2890, 1994; Marshall *et al.*, *J. Immunol.* 152:4946, 1994), ELISA systems (*e.g.*, Reay *et al.*, *EMBO J.* 11:2829, 1992), surface plasmon resonance (*e.g.*, Khilko *et al.*, *J. Biol. Chem.* 268:15425, 1993); high flux soluble phase assays (Hammer *et al.*, *J. Exp. Med.* 180:2353, 1994), and measurement of class I MHC stabilization or assembly (*e.g.*, Ljunggren *et al.*, *Nature* 346:476, 1990; Schumacher *et al.*, *Cell* 62:563, 1990; Townsend *et al.*, *Cell* 62:285, 1990; Parker *et al.*, *J. Immunol.* 149:1896, 1992).

As used herein, "high affinity" with respect to HLA class I molecules is defined as binding with an IC_{50}, or K_D value, of 50 nM or less; "intermediate affinity" is binding with an IC_{50} or K_D value of between about 50 and about 500 nM. "High affinity" with respect to binding to HLA class II molecules is defined as binding with an IC_{50} or K_D value of 100 nM or less; "intermediate affinity" is binding with an IC_{50} or K_D value of between about 100 and about 1000 nM.

The terms "identical" or percent "identity," in the context of two or more peptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues that are the same, when compared and aligned for maximum correspondence over a comparison window, as measured using a sequence comparison algorithm or by manual alignment and visual inspection.

An "immunogenic peptide" or "peptide epitope" is a peptide that comprises an allele-specific motif or supermotif such that the peptide will bind an HLA molecule and induce a CTL and/or HTL response.

Thus, immunogenic peptides of the invention are capable of binding to an appropriate HLA molecule and thereafter inducing a cytotoxic T cell response, or a helper T cell response, to the antigen from which the immunogenic peptide is derived.

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany the material as it is found in its native state. Thus, isolated peptides in accordance with the invention preferably do not contain materials normally associated with the peptides in their *in situ* environment.

"Link" or "join" refers to any method known in the art for functionally connecting peptides, including, without limitation, recombinant fusion, covalent bonding, disulfide bonding, ionic bonding, hydrogen bonding, and electrostatic bonding.

"Major Histocompatibility Complex" or "MHC" is a cluster of genes that plays a role in control of the cellular interactions responsible for physiologic immune responses. In humans, the MHC complex is also known as the HLA complex. For a detailed description of the MHC and HLA complexes, see, Paul, FUNDAMENTAL IMMUNOLOGY, 3RD ED., Raven Press, New York, 1993.

The term "motif" refers to the pattern of residues in a peptide of defined length, usually a peptide of from about 8 to about 13 amino acids for a class I HLA motif and from about 6 to about 25 amino acids for a class II HLA motif, which is recognized by a particular HLA molecule. Peptide motifs are typically different for each protein encoded by each human HLA allele and differ in the pattern of the primary and secondary anchor residues.

A "negative binding residue" or "deleterious residue" is an amino acid which, if present at certain positions (typically not primary anchor positions) in a peptide epitope, results in decreased binding affinity of the peptide for the peptide's corresponding HLA molecule.

The term "peptide" is used interchangeably with "oligopeptide" in the present specification to designate a series of residues, typically L-amino acids, connected one to the other, typically by peptide bonds between the α-amino and carboxyl groups of adjacent amino acids. The preferred CTL-inducing peptides of the invention are 13 residues or less in length and usually consist of between about 8 and about 11 residues, preferably 9 or 10 residues. The preferred HTL-inducing oligopeptides are less than about 50 residues in length and usually consist of between about 6 and about 30 residues, more usually between about 12 and 25, and often between about 15 and 20 residues.

"Pharmaceutically acceptable" refers to a generally non-toxic, inert, and/or physiologically compatible composition.

A "pharmaceutical excipient" comprises a material such as an adjuvant, a carrier, pH-adjusting and buffering agents, tonicity adjusting agents, wetting agents, preservative, and the like.

A "primary anchor residue" is an amino acid at a specific position along a peptide sequence which is understood to provide a contact point between the immunogenic peptide and the HLA molecule. One to three, usually two, primary anchor residues within a peptide of defined length generally defines a "motif" for an immunogenic peptide. These residues are understood to fit in close contact with peptide binding grooves of an HLA molecule, with their side chains buried in specific pockets of the binding grooves themselves. In one embodiment, for example, the primary anchor residues are located at position 2 (from the amino terminal position) and at the carboxyl terminal position of a 9-residue peptide epitope in

accordance with the invention. The primary anchor positions for each motif and supermotif are set forth in Table 1. For example, analog peptides can be created by altering the presence or absence of particular residues in these primary anchor positions. Such analogs are used to modulate the binding affinity of a peptide comprising a particular motif or supermotif.

"Promiscuous recognition" is where a distinct peptide is recognized by the same T cell clone in the context of various HLA molecules. Promiscuous recognition or binding is synonymous with cross-reactive binding.

A "protective immune response" or "therapeutic immune response" refers to a CTL and/or an HTL response to an antigen derived from an infectious agent or a tumor antigen, which prevents or at least partially arrests disease symptoms or progression. The immune response may also include an antibody response which has been facilitated by the stimulation of helper T cells.

The term "residue" refers to an amino acid or amino acid mimetic incorporated into an oligopeptide by an amide bond or amide bond mimetic.

A "secondary anchor residue" is an amino acid at a position other than a primary anchor position in a peptide which may influence peptide binding. A secondary anchor residue occurs at a significantly higher frequency amongst bound peptides than would be expected by random distribution of amino acids at one position. The secondary anchor residues are said to occur at "secondary anchor positions." A secondary anchor residue can be identified as a residue which is present at a higher frequency among high or intermediate affinity binding peptides, or a residue otherwise associated with high or intermediate affinity binding. For example, analog peptides can be created by altering the presence or absence of particular residues in these secondary anchor positions. Such analogs are used to finely modulate the binding affinity of a peptide comprising a particular motif or supermotif.

A "subdominant epitope" is an epitope which evokes little or no response upon immunization with whole antigens which comprise the epitope, but for which a response can be obtained by immunization with an isolated peptide, and this response (unlike the case of cryptic epitopes) is detected when whole protein is used to recall the response *in vitro* or *in vivo*.

A "supermotif" is a peptide binding specificity shared by HLA molecules encoded by two or more HLA alleles. Preferably, a supermotif-bearing peptide is recognized with high or intermediate affinity (as defined herein) by two or more HLA molecules.

"Synthetic peptide" refers to a peptide that is man-made using such methods as chemical synthesis or recombinant DNA technology.

As used herein, a "vaccine" is a composition that contains one or more peptides of the invention. There are numerous embodiments of vaccines in accordance with the invention, such as by a cocktail of one or more peptides; one or more epitopes of the invention comprised by a polyepitopic peptide; or nucleic acids that encode such peptides or polypeptides, *e.g.*, a minigene that encodes a polyepitopic peptide. The "one or more peptides" can include any whole unit integer from 1-150, *e.g.*, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 , 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, or 150 or more peptides of the invention. The peptides or polypeptides can optionally be modified, such as by lipidation, addition of targeting or other sequences. HLA class I-binding peptides of the invention can be

admixed with, or linked to, HLA class II-binding peptides, to facilitate activation of both cytotoxic T lymphocytes and helper T lymphocytes. Vaccines can also comprise peptide-pulsed antigen presenting cells, *e.g.*, dendritic cells.

The nomenclature used to describe peptide compounds follows the conventional practice wherein the amino group is presented to the left (the N-terminus) and the carboxyl group to the right (the C-terminus) of each amino acid residue. When amino acid residue positions are referred to in a peptide epitope they are numbered in an amino to carboxyl direction with position one being the position closest to the amino terminal end of the epitope, or the peptide or protein of which it may be a part. In the formulae representing selected specific embodiments of the present invention, the amino- and carboxyl-terminal groups, although not specifically shown, are in the form they would assume at physiologic pH values, unless otherwise specified. In the amino acid structure formulae, each residue is generally represented by standard three letter or single letter designations. The L-form of an amino acid residue is represented by a capital single letter or a capital first letter of a three-letter symbol, and the D-form for those amino acids having D-forms is represented by a lower case single letter or a lower case three letter symbol. Glycine has no asymmetric carbon atom and is simply referred to as "Gly" or G. The amino acid sequences of peptides set forth herein are generally designated using the standard single letter symbol. (A, Alanine; C, Cysteine; D, Aspartic Acid; E, Glutamic Acid; F, Phenylalanine; G, Glycine; H, Histidine; I, Isoleucine; K, Lysine; L, Leucine; M, Methionine; N, Asparagine; P, Proline; Q, Glutamine; R, Arginine; S, Serine; T, Threonine; V, Valine; W, Tryptophan; and Y, Tyrosine.) In addition to these symbols, "B"in the single letter abbreviations used herein designates α-amino butyric acid.

IV.B. Stimulation of CTL and HTL responses

The mechanism by which T cells recognize antigens has been delineated during the past ten years. Based on our understanding of the immune system we have developed efficacious peptide epitope vaccine compositions that can induce a therapeutic or prophylactic immune response to a TAA in a broad population. For an understanding of the value and efficacy of the claimed compositions, a brief review of immunology-related technology is provided. The review is intended to disclose the presently understood state of the art as of the filing date of the present application. Information is included in this section which was generated subsequent to the priority date of this application. Accordingly, information in this section is not intended, in any way, to delineate the priority date for the invention.

A complex of an HLA molecule and a peptidic antigen acts as the ligand recognized by HLA-restricted T cells (Buus, S. *et al.*, *Cell* 47:1071, 1986; Babbitt, B. P. *et al.*, *Nature* 317:359, 1985; Townsend, A. and Bodmer, H., *Annu. Rev. Immunol.* 7:601, 1989; Germain, R. N., *Annu. Rev. Immunol.* 11:403, 1993). Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues that correspond to motifs required for specific binding to HLA antigen molecules have been identified and are described herein and are set forth in Tables I, II, and III (*see* also, *e.g.*, Southwood, *et al.*, *J. Immunol.* 160:3363, 1998; Rammensee, *et al.*, *Immunogenetics* 41:178, 1995; Rammensee *et al.*, SYFPEITHI, access via web at : http://134.2.96.221/scripts.hlaserver.dll/home.htm; Sette, A. and Sidney, J. *Curr. Opin. Immunol.* 10:478, 1998; Engelhard, V. H., *Curr. Opin. Immunol.* 6:13, 1994; Sette, A. and Grey, H. M., *Curr. Opin. Immunol.*

4:79, 1992; Sinigaglia, F. and Hammer, J. *Curr. Biol.* 6:52, 1994; Ruppert *et al.*, *Cell* 74:929-937, 1993; Kondo *et al.*, *J. Immunol.* 155:4307-4312, 1995; Sidney *et al.*, *J. Immunol.* 157:3480-3490, 1996; Sidney *et al.*, *Human Immunol.* 45:79-93, 1996; Sette, A. and Sidney, J. *Immunogenetics 1999 Nov;50(3-4):201-12,* Review).

Furthermore, x-ray crystallographic analysis of HLA-peptide complexes has revealed pockets within the peptide binding cleft of HLA molecules which accommodate, in an allele-specific mode, residues borne by peptide ligands; these residues in turn determine the HLA binding capacity of the peptides in which they are present. (*See, e.g.*, Madden, D.R. *Annu. Rev. Immunol.* 13:587, 1995; Smith, *et al.*, *Immunity* 4:203, 1996; Fremont *et al.*, *Immunity* 8:305, 1998; Stern *et al.*, *Structure* 2:245, 1994; Jones, E.Y. *Curr. Opin. Immunol.* 9:75, 1997; Brown, J. H. *et al.*, *Nature* 364:33, 1993; Guo, H. C. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:8053, 1993; Guo, H. C. *et al.*, *Nature* 360:364, 1992; Silver, M. L. *et al.*, *Nature* 360:367, 1992; Matsumura, M. *et al.*, *Science* 257:927, 1992; Madden *et al.*, *Cell* 70:1035, 1992; Fremont, D. H. *et al.*, *Science* 257:919, 1992; Saper, M. A., Bjorkman, P. J. and Wiley, D. C., *J. Mol. Biol.* 219:277, 1991.)

Accordingly, the definition of class I and class II allele-specific HLA binding motifs, or class I or class II supermotifs allows identification of regions within a protein that have the potential of binding particular HLA molecules.

The present inventors have found that the correlation of binding affinity with immunogenicity, which is disclosed herein, is an important factor to be considered when evaluating candidate peptides. Thus, by a combination of motif searches and HLA-peptide binding assays, candidates for epitope-based vaccines have been identified. After determining their binding affinity, additional confirmatory work can be performed to select, amongst these vaccine candidates, epitopes with preferred characteristics in terms of population coverage, antigenicity, and immunogenicity.

Various strategies can be utilized to evaluate immunogenicity, including:

1) Evaluation of primary T cell cultures from normal individuals (*see, e.g.*, Wentworth, P. A. *et al.*, *Mol. Immunol.* 32:603, 1995; Celis, E. *et al.*, *Proc. Natl. Acad. Sci. USA* 91:2105, 1994; Tsai, V. *et al.*, *J. Immunol.* 158:1796, 1997; Kawashima, I. *et al.*, *Human Immunol.* 59:1, 1998); This procedure involves the stimulation of peripheral blood lymphocytes (PBL) from normal subjects with a test peptide in the presence of antigen presenting cells *in vitro* over a period of several weeks. T cells specific for the peptide become activated during this time and are detected using, *e.g.*, a ^{51}Cr-release assay involving peptide sensitized target cells.

2) Immunization of HLA transgenic mice (*see, e.g.*, Wentworth, P. A. *et al.*, *J. Immunol.* 26:97, 1996; Wentworth, P. A. *et al.*, *Int. Immunol.* 8:651, 1996; Alexander, J. *et al.*, *J. Immunol.* 159:4753, 1997); In this method, peptides in incomplete Freund's adjuvant are administered subcutaneously to HLA transgenic mice. Several weeks following immunization, splenocytes are removed and cultured *in vitro* in the presence of test peptide for approximately one week. Peptide-specific T cells are detected using, *e.g.*, a ^{51}Cr-release assay involving peptide sensitized target cells and target cells expressing endogenously generated antigen.

3) Demonstration of recall T cell responses from patients who have been effectively vaccinated or who have a tumor; (*see, e.g.*, Rehermann, B. *et al.*, *J. Exp. Med.* 181:1047, 1995; Doolan, D. L. *et al.*, *Immunity* 7:97, 1997; Bertoni, R. *et al.*, *J. Clin. Invest.* 100:503, 1997; Threlkeld, S. C. *et al.*, *J. Immunol.*

159:1648, 1997; Diepolder, H. M. *et al.*, *J. Virol.* 71:6011, 1997; Tsang *et al.*, *J. Natl. Cancer Inst.* 87:982-990, 1995; Disis *et al.*, *J. Immunol.* 156:3151-3158, 1996). In applying this strategy, recall responses are detected by culturing PBL from patients with cancer who have generated an immune response "naturally", or from patients who were vaccinated with tumor antigen vaccines. PBL from subjects are cultured *in vitro* for 1-2 weeks in the presence of test peptide plus antigen presenting cells (APC) to allow activation of "memory" T cells, as compared to "naive" T cells. At the end of the culture period, T cell activity is detected using assays for T cell activity including ^{51}Cr release involving peptide-sensitized targets, T cell proliferation, or lymphokine release.

The following describes the peptide epitopes and corresponding nucleic acids of the invention.

IV.C. Binding Affinity of Peptide Epitopes for HLA Molecules

As indicated herein, the large degree of HLA polymorphism is an important factor to be taken into account with the epitope-based approach to vaccine development. To address this factor, epitope selection encompassing identification of peptides capable of binding at high or intermediate affinity to multiple HLA molecules is preferably utilized, most preferably these epitopes bind at high or intermediate affinity to two or more allele-specific HLA molecules.

CTL-inducing peptides of interest for vaccine compositions preferably include those that have an IC_{50} or binding affinity value for class I HLA molecules of 500 nM or better (*i.e.*, the value is $\leq$ 500 nM). HTL-inducing peptides preferably include those that have an IC_{50} or binding affinity value for class II HLA molecules of 1000 nM or better, (*i.e.*, the value is $\leq$ 1,000 nM). For example, peptide binding is assessed by testing the capacity of a candidate peptide to bind to a purified HLA molecule *in vitro*. Peptides exhibiting high or intermediate affinity are then considered for further analysis. Selected peptides are tested on other members of the supertype family. In preferred embodiments, peptides that exhibit cross-reactive binding are then used in cellular screening analyses or vaccines.

As disclosed herein, higher HLA binding affinity is correlated with greater immunogenicity. Greater immunogenicity can be manifested in several different ways. Immunogenicity corresponds to whether an immune response is elicited at all, and to the vigor of any particular response, as well as to the extent of a population in which a response is elicited. For example, a peptide might elicit an immune response in a diverse array of the population, yet in no instance produce a vigorous response. Moreover, higher binding affinity peptides lead to more vigorous immunogenic responses. As a result, less peptide is required to elicit a similar biological effect if a high or intermediate affinity binding peptide is used. Thus, in preferred embodiments of the invention, high or intermediate affinity binding epitopes are particularly useful.

The relationship between binding affinity for HLA class I molecules and immunogenicity of discrete peptide epitopes on bound antigens has been determined for the first time in the art by the present inventors. The correlation between binding affinity and immunogenicity was analyzed in two different experimental approaches (*see, e.g.*, Sette, *et al.*, *J. Immunol.* 153:5586-5592, 1994). In the first approach, the immunogenicity of potential epitopes ranging in HLA binding affinity over a 10,000-fold range was analyzed in HLA-A*0201 transgenic mice. In the second approach, the antigenicity of approximately 100 different hepatitis B virus (HBV)-derived potential epitopes, all carrying A*0201 binding motifs, was

assessed by using PBL from acute hepatitis patients. Pursuant to these approaches, it was determined that an affinity threshold value of approximately 500 nM (preferably 50 nM or less) determines the capacity of a peptide epitope to elicit a CTL response. These data are true for class I binding affinity measurements for naturally processed peptides and for synthesized T cell epitopes. These data also indicate the important role of determinant selection in the shaping of T cell responses (*see, e.g.*, Schaeffer *et al., Proc. Natl. Acad. Sci. USA* 86:4649-4653, 1989).

An affinity threshold associated with immunogenicity in the context of HLA class II DR molecules has also been delineated (*see, e.g.*, Southwood *et al. J. Immunology* 160:3363-3373,1998, and co-pending U.S.S.N. 09/009,953 filed 1/21/98). In order to define a biologically significant threshold of DR binding affinity, a database of the binding affinities of 32 DR-restricted epitopes for their restricting element (*i.e.*, the HLA molecule that binds the motif) was compiled. In approximately half of the cases (15 of 32 epitopes), DR restriction was associated with high binding affinities, *i.e.* binding affinity values of 100 nM or less. In the other half of the cases (16 of 32), DR restriction was associated with intermediate affinity (binding affinity values in the 100-1000 nM range). In only one of 32 cases was DR restriction associated with an IC_{50} of 1000 nM or greater. Thus, 1000 nM can be defined as an affinity threshold associated with immunogenicity in the context of DR molecules.

In the case of tumor-associated antigens, many CTL peptide epitopes that have been shown to induce CTL that lyse peptide-pulsed target cells and tumor cell targets endogenously expressing the epitope exhibit binding affinity or IC_{50} values of 200 nM or less. In a study that evaluated the association of binding affinity and immunogenicity of such TAA epitopes, 100% (10/10) of the high binders, *i.e.*, peptide epitopes binding at an affinity of 50 nM or less, were immunogenic and 80% (8/10) of them elicited CTLs that specifically recognized tumor cells. In the 51 to 200 nM range, very similar figures were obtained. CTL inductions positive for peptide and tumor cells were noted for 86% (6/7) and 71% (5/7) of the peptides, respectively. In the 201-500 nM range, most peptides (4/5 wildtype) were positive for induction of CTL recognizing wildtype peptide, but tumor recognition was not detected.

The binding affinity of peptides for HLA molecules can be determined as described in Example 1, below.

IV.D. Peptide Epitope Binding Motifs and Supermotifs

Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues required for allele-specific binding to HLA molecules have been identified. The presence of these residues correlates with binding affinity for HLA molecules. The identification of motifs and/or supermotifs that correlate with high and intermediate affinity binding is an important issue with respect to the identification of immunogenic peptide epitopes for the inclusion in a vaccine. Kast *et al.* (*J. Immunol.* 152:3904-3912, 1994) have shown that motif-bearing peptides account for 90% of the epitopes that bind to allele-specific HLA class I molecules. In this study all possible peptides of 9 amino acids in length and overlapping by eight amino acids (240 peptides), which cover the entire sequence of the E6 and E7 proteins of human papillomavirus type 16, were evaluated for binding to five allele-specific HLA molecules that are expressed at high frequency among different ethnic groups. This unbiased set of peptides allowed an evaluation of the predictive value of HLA class I motifs.

From the set of 240 peptides, 22 peptides were identified that bound to an allele-specific HLA molecule with high or intermediate affinity. Of these 22 peptides, 20 (*i.e.* 91%) were motif-bearing. Thus, this study demonstrates the value of motifs for the identification of peptide epitopes for inclusion in a vaccine: application of motif-based identification techniques will identify about 90% of the potential epitopes in a target antigen protein sequence.

Such peptide epitopes are identified in the Tables described below.

Peptides of the present invention also comprise epitopes that bind to MHC class II DR molecules. A greater degree of heterogeneity in both size and binding frame position of the motif, relative to the N and C termini of the peptide, exists for class II peptide ligands. This increased heterogeneity of HLA class II peptide ligands is due to the structure of the binding groove of the HLA class II molecule which, unlike its class I counterpart, is open at both ends. Crystallographic analysis of HLA class II DRB*0101-peptide complexes showed that the major energy of binding is contributed by peptide residues complexed with complementary pockets on the DRB*0101 molecules. An important anchor residue engages the deepest hydrophobic pocket (*see, e.g.*, Madden, D.R. *Ann. Rev. Immunol.* 13:587, 1995) and is referred to as position 1 (P1). P1 may represent the N-terminal residue of a class II binding peptide epitope, but more typically is flanked towards the N-terminus by one or more residues. Other studies have also pointed to an important role for the peptide residue in the 6th position towards the C-terminus, relative to P1, for binding to various DR molecules.

In the past few years evidence has accumulated to demonstrate that a large fraction of HLA class I and class II molecules can be classified into a relatively few supertypes, each characterized by largely overlapping peptide binding repertoires, and consensus structures of the main peptide binding pockets. Thus, peptides of the present invention are identified by any one of several HLA-specific amino acid motifs (*see, e.g.*, Tables I-III), or if the presence of the motif corresponds to the ability to bind several allele-specific HLA molecules, a supermotif. The HLA molecules that bind to peptides that possess a particular amino acid supermotif are collectively referred to as an HLA "supertype."

The peptide motifs and supermotifs described below, and summarized in Tables I-III, provide guidance for the identification and use of peptide epitopes in accordance with the invention.

Examples of peptide epitopes bearing a respective supermotif or motif are included in Tables as designated in the description of each motif or supermotif below. The Tables include a binding affinity ratio listing for some of the peptide epitopes. The ratio may be converted to IC_{50} by using the following formula: IC_{50} of the standard peptide/ratio = IC_{50} of the test peptide (*i.e.*, the peptide epitope). The IC_{50} values of standard peptides used to determine binding affinities for Class I peptides are shown in Table IV. The IC_{50} values of standard peptides used to determine binding affinities for Class II peptides are shown in Table V. The peptides used as standards for the binding assays described herein are examples of standards; alternative standard peptides can also be used when performing binding studies.

To obtain the peptide epitope sequences listed in each of Tables VII-XX, the amino acid sequence of HER2/neu was evaluated for the presence of the designated supermotif or motif, *i.e.*, the amino acid sequence was searched for the presence of the primary anchor residues as set out in Table I (for Class I motifs) or Table III (for Class II motifs) for each respective motif or supermotif.

In the Tables, motif- and/or supermotif-bearing epitopes in the HER2/neu sequence are indicated by position number and length of the epitope with reference to the HER2/neu sequence and numbering provided below. The "pos" (position) column designates the amino acid position in the HER2/neu protein sequence that corresponds to the first amino acid residue of the epitope. The "number of amino acids" indicates the number of residues in the epitope sequence and hence the length of the epitope. For example, the first peptide epitope listed in Table VII is a sequence of 8 residues in length starting at position 66. Accordingly, the amino acid sequence of the epitope is PTNASLSF.

Binding data presented in Tables VII-XX is expressed as a relative binding ratio, *supra*.

HER2/neu amino acid sequence

```
1 MELAALCRWG LLLALLPPGA ASTQVCTGTD MKLRLPASPE THLDMLRHLY QGCQVVQGNL   60
  ELTYLPTNAS LSFLQDIQEV QGYVLIAHNQ VRQVPLQRLR IVRGTQLFED NYALAVLDNG  120
  DPLNNTTPVT GASPGGLREL QLRSLTEILK GGVLIQRNPQ LCYQDTILWK DIFHKNNQLA  180
  LTLIDTNRSR ACHPCSPMCK GSRCWGESSE DCQSLTRTVC AGGCARCKGP LPTDCCHEQC  240
  AAGCTGPKHS DCLACLHFNH SGICELHCPA LVTYNTDTFE SMPNPEGRYT FGASCVTACP  300
  YNYLSTDVGS CTLVCPLHNQ EVTAEDGTQR CEKCSKPCAR VCYGLGMEHL REVRAVTSAN  360
  IQEFAGCKKI FGSLAFLPES FDGDPASNTA PLQPEQLQVF ETLEEITGYL YISAWPDSLP  420
  DLSVFQNLQV IRGRILHNGA YSLTLQGLGI SWLGLRSLRE LGSGLALIHH NTHLCFVHTV  480
  PWDQLFRNPH QALLHTANRP EDECVGEGLA CHQLCARGHC WGPGPTQCVN CSQFLRGQEC  540
  VEECRVLQGL PREYVNARHC LPCHPECQPQ NGSVTCFGPE ADQCVACAHY KDPPFCVARC  600
  PSGVKPDLSY MPIWKFPDEE GACQPCPINC THSCVDLDDK GCPAEQRASP LTSIISAVVG  660
  ILLVVVLGVV FGILIKRRQQ KIRKYTMRRL LQETELVEPL TPSGAMPNQA QMRILKETEL  720
  RKVKVLGSGA FGTVYKGIWI PDGENVKIPV AIKVLRENTS PKANKEILDE AYVMAGVGSP  780
  YVSRLLGICL TSTVQLVTQL MPYGCLLDHV RENRGRLGSQ DLLNWCMQIA KGMSYLEDVR  840
  LVHRDLAARN VLVKSPNHVK ITDFGLARLL DIDETEYHAD GGKVPIKWMA LESILRRRFT  900
  HQSDVWSYGV TVWELMTFGA KPYDGIPARE IPDLLEKGER LPQPPICTID VYMIMVKCWM  960
  IDSECRPRFR ELVSEFSRMA RDPQRFVVIQ NEDLGPASPL DSTFYRSLLE DDDMGDLVDA 1020
  EEYLVPQQGF FCPDPAPGAG GMVHHRHRSS STRSGGGDLT LGLEPSEEEA PRSPLAPSEG 1080
  AGSDVFDGDL GMGAAKGLQS LPTHDPSPLQ RYSEDPTVPL PSETDGYVAP LTCSPQPEYV 1140
  NQPDVRPQPP SPREGPLPAA RPAGATLERP KTLSPGKNGV VKDVFAFGGA VENPEYLTPQ 1200
  GGAAPQPHPP PAFSPAFDNL YYWDQDPPER GAPPSTFKGT PTAENPEYLG LDVPV  1255
```

HLA Class I Motifs Indicative of CTL Inducing Peptide Epitopes:

The primary anchor residues of the HLA class I peptide epitope supermotifs and motifs delineated below are summarized in Table I. The HLA class I motifs set out in Table I(a) are those most particularly relevant to the invention claimed here. Primary and secondary anchor positions are summarized in Table II. Allele-specific HLA molecules that comprise HLA class I supertype families are listed in Table VI. In some cases, peptide epitopes may be listed in both a motif and a supermotif Table. The relationship of a particular motif and respective supermotif is indicated in the description of the individual motifs.

IV.D.1. HLA-A1 supermotif

The HLA-A1 supermotif is characterized by the presence in peptide ligands of a small (T or S) or hydrophobic (L, I, V, or M) primary anchor residue in position 2, and an aromatic (Y, F, or W) primary anchor residue at the C-terminal position of the epitope. The corresponding family of HLA molecules that bind to the A1 supermotif (*i.e.*, the HLA-A1 supertype) is comprised of at least: A*0101, A*2601, A*2602, A*2501, and A*3201 (*see, e.g.*, DiBrino, M. *et al.*, *J. Immunol.* 151:5930, 1993; DiBrino, M. *et al.*, *J. Immunol.* 152:620, 1994; Kondo, A. *et al.*, *Immunogenetics* 45:249, 1997). Other allele-specific HLA molecules predicted to be members of the A1 superfamily are shown in Table VI. Peptides binding to each of the individual HLA proteins can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the A1 supermotif are set forth in Table VII.

IV.D.2. HLA-A2 supermotif

Primary anchor specificities for allele-specific HLA-A2.1 molecules (*see, e.g.*, Falk *et al.*, *Nature* 351:290-296, 1991; Hunt *et al.*, *Science* 255:1261-1263, 1992; Parker *et al.*, *J. Immunol.* 149:3580-3587, 1992; Ruppert *et al.*, *Cell* 74:929-937, 1993) and cross-reactive binding among HLA-A2 and -A28 molecules have been described. (*See, e.g.*, Fruci *et al.*, *Human Immunol.* 38:187-192, 1993; Tanigaki *et al.*, *Human Immunol.* 39:155-162, 1994; Del Guercio *et al.*, *J. Immunol.* 154:685-693, 1995; Kast *et al.*, *J. Immunol.* 152:3904-3912, 1994 for reviews of relevant data.) These primary anchor residues define the HLA-A2 supermotif; which presence in peptide ligands corresponds to the ability to bind several different HLA-A2 and -A28 molecules. The HLA-A2 supermotif comprises peptide ligands with L, I, V, M, A, T, or Q as a primary anchor residue at position 2 and L, I, V, M, A, or T as a primary anchor residue at the C-terminal position of the epitope.

The corresponding family of HLA molecules (*i.e.*, the HLA-A2 supertype that binds these peptides) is comprised of at least: A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*0209, A*0214, A*6802, and A*6901. Other allele-specific HLA molecules predicted to be members of the A2 superfamily are shown in Table VI. As explained in detail below, binding to each of the individual allele-specific HLA molecules can be modulated by substitutions at the primary anchor and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise an A2 supermotif are set forth in Table VIII. The motifs comprising the primary anchor residues V, A, T, or Q at position 2 and L, I, V, A, or T at the C-terminal position are those most particularly relevant to the invention claimed herein.

IV.D.3. HLA-A3 supermotif

The HLA-A3 supermotif is characterized by the presence in peptide ligands of A, L, I, V, M, S, or, T as a primary anchor at position 2, and a positively charged residue, R or K, at the C-terminal position of the epitope, *e.g.*, in position 9 of 9-mers (*see, e.g.*, Sidney *et al.*, *Hum. Immunol.* 45:79, 1996). Exemplary members of the corresponding family of HLA molecules (the HLA-A3 supertype) that bind the A3 supermotif include at least: A*0301, A*1101, A*3101, A*3301, and A*6801. Other allele-specific HLA molecules predicted to be members of the A3 supertype are shown in Table VI. As explained in detail below, peptide binding to each of the individual allele-specific HLA proteins can be modulated by substitutions of amino acids at the primary and/or secondary anchor positions of the peptide, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the A3 supermotif are set forth in Table IX.

IV.D.4. HLA-A24 supermotif

The HLA-A24 supermotif is characterized by the presence in peptide ligands of an aromatic (F, W, or Y) or hydrophobic aliphatic (L, I, V, M, or T) residue as a primary anchor in position 2, and Y, F, W, L, I, or M as primary anchor at the C-terminal position of the epitope (*see, e.g.*, Sette and Sidney, *Immunogenetics 1999 Nov;50(3-4):201-12,* Review). The corresponding family of HLA molecules that bind to the A24 supermotif (*i.e.*, the A24 supertype) includes at least: A*2402, A*3001, and A*2301. Other allele-specific HLA molecules predicted to be members of the A24 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the A24 supermotif are set forth in Table X.

IV.D.5. HLA-B7 supermotif

The HLA-B7 supermotif is characterized by peptides bearing proline in position 2 as a primary anchor, and a hydrophobic or aliphatic amino acid (L, I, V, M, A, F, W, or Y) as the primary anchor at the C-terminal position of the epitope. The corresponding family of HLA molecules that bind the B7 supermotif (*i.e.*, the HLA-B7 supertype) is comprised of at least twenty six HLA-B proteins comprising at least: B*0702, B*0703, B*0704, B*0705, B*1508, B*3501, B*3502, B*3503, B*3504, B*3505, B*3506, B*3507, B*3508, B*5101, B*5102, B*5103, B*5104, B*5105, B*5301, B*5401, B*5501, B*5502, B*5601, B*5602, B*6701, and B*7801 (*see, e.g.*, Sidney, *et al., J. Immunol.* 154:247, 1995; Barber, *et al., Curr. Biol.* 5:179, 1995; Hill, *et al., Nature* 360:434, 1992; Rammensee, *et al., Immunogenetics* 41:178, 1995 for reviews of relevant data). Other allele-specific HLA molecules predicted to be members of the B7 supertype are shown in Table VI. As explained in detail below, peptide binding to each of the individual allele-specific HLA proteins can be modulated by substitutions at the primary and/or secondary anchor positions of the peptide, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B7 supermotif are set forth in Table XI.

IV.D.6. HLA-B27 supermotif

The HLA-B27 supermotif is characterized by the presence in peptide ligands of a positively charged (R, H, or K) residue as a primary anchor at position 2, and a hydrophobic (F, Y, L, W, M, I, A, or V) residue as a primary anchor at the C-terminal position of the epitope (*see, e.g.*, Sidney and Sette, *Immunogenetics 1999 Nov;50(3-4):201-12,* Review). Exemplary members of the corresponding family of HLA molecules that bind to the B27 supermotif (*i.e.*, the B27 supertype) include at least B*1401, B*1402, B*1509, B*2702, B*2703, B*2704, B*2705, B*2706, B*3801, B*3901, B*3902, and B*7301. Other allele-specific HLA molecules predicted to be members of the B27 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B27 supermotif are set forth in Table XII.

IV.D.7. HLA-B44 supermotif

The HLA-B44 supermotif is characterized by the presence in peptide ligands of negatively charged (D or E) residues as a primary anchor in position 2, and hydrophobic residues (F, W, Y, L, I, M, V, or A) as a primary anchor at the C-terminal position of the epitope (*see, e.g.*, Sidney et al., *Immunol. Today* 17:261, 1996). Exemplary members of the corresponding family of HLA molecules that bind to the B44 supermotif (*i.e.*, the B44 supertype) include at least: B*1801, B*1802, B*3701, B*4001, B*4002, B*4006, B*4402, B*4403, and B*4404. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions; preferably choosing respective residues specified for the supermotif.

IV.D.8. HLA-B58 supermotif

The HLA-B58 supermotif is characterized by the presence in peptide ligands of a small aliphatic residue (A, S, or T) as a primary anchor residue at position 2, and an aromatic or hydrophobic residue (F, W, Y, L, I, V, M, or A) as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.*, Sidney and Sette, *Immunogenetics 1999 Nov;50(3-4):201-12,* Review). Exemplary members of the corresponding family of HLA molecules that bind to the B58 supermotif (*i.e.*, the B58 supertype) include at least: B*1516, B*1517, B*5701, B*5702, and B*5801. Other allele-specific HLA molecules predicted to be members of the B58 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B58 supermotif are set forth in Table XIII.

IV.D.9. HLA-B62 supermotif

The HLA-B62 supermotif is characterized by the presence in peptide ligands of the polar aliphatic residue Q or a hydrophobic aliphatic residue (L, V, M, I, or P) as a primary anchor in position 2, and a hydrophobic residue (F, W, Y, M, I, V, L, or A) as a primary anchor at the C-terminal position of the

epitope (*see, e.g.*, Sidney and Sette, *Immunogenetics 1999 Nov;50(3-4):201-12,* Review). Exemplary members of the corresponding family of HLA molecules that bind to the B62 supermotif (*i.e.*, the B62 supertype) include at least: B*1501, B*1502, B*1513, and B5201. Other allele-specific HLA molecules predicted to be members of the B62 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B62 supermotif are set forth in Table XIV.

IV.D.10. HLA-A1 motif

The HLA-A1 motif is characterized by the presence in peptide ligands of T, S, or M as a primary anchor residue at position 2 and the presence of Y as a primary anchor residue at the C-terminal position of the epitope. An alternative allele-specific A1 motif is characterized by a primary anchor residue at position 3 rather than position 2. This motif is characterized by the presence of D, E, A, or S as a primary anchor residue in position 3, and a Y as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.*, DiBrino *et al.*, J. Immunol., 152:620, 1994; Kondo *et al.*, *Immunogenetics* 45:249, 1997; and Kubo *et al.*, *J. Immunol.* 152:3913, 1994 for reviews of relevant data). Peptide binding to HLA-A1 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise either A1 motif are set forth in Table XV. Those epitopes comprising T, S, or M at position 2 and Y at the C-terminal position are also included in the listing of HLA-A1 supermotif-bearing peptide epitopes listed in Table VII, as these residues are a subset of the A1 supermotif primary anchors.

IV.D.11. HLA-A*0201 motif

An HLA-A2*0201 motif was determined to be characterized by the presence in peptide ligands of L or M as a primary anchor residue in position 2, and L or V as a primary anchor residue at the C-terminal position of a 9-residue peptide (*see, e.g.*, Falk *et al.*, *Nature* 351:290-296, 1991) and was further found to comprise an I at position 2 and I or A at the C-terminal position of a nine amino acid peptide (*see, e.g.*, Hunt *et al.*, Science 255:1261-1263, March 6, 1992; Parker *et al.*, *J. Immunol.* 149:3580-3587, 1992). The A*0201 allele-specific motif has also been defined by the present inventors to additionally comprise V, A, T, or Q as a primary anchor residue at position 2, and M or T as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.*, Kast *et al.*, *J. Immunol.* 152:3904-3912, 1994). Thus, the HLA-A*0201 motif comprises peptide ligands with L, I, V, M, A, T, or Q as primary anchor residues at position 2 and L, I, V, M, A, or T as a primary anchor residue at the C-terminal position of the epitope. The preferred and tolerated residues that characterize the primary anchor positions of the HLA-A*0201 motif are identical to the residues describing the A2 supermotif. (For reviews of relevant data, *see, e.g.*, del Guercio *et al.*, *J. Immunol.* 154:685-693, 1995; Ruppert *et al.*, *Cell* 74:929-937, 1993; Sidney *et al.*, *Immunol. Today* 17:261-266, 1996; Sette and Sidney, *Curr. Opin. in Immunol.* 10:478-482, 1998). Secondary anchor residues that characterize the A*0201 motif have additionally been defined (*see, e.g.*, Ruppert *et al.*, *Cell* 74:929-937, 1993). These are shown in Table II. Peptide binding to HLA-A*0201 molecules can be modulated by

substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise an A*0201 motif are set forth in Table VIII. The A*0201 motifs comprising the primary anchor residues V, A, T, or Q at position 2 and L, I, V, A, or T at the C-terminal position are those most particularly relevant to the invention claimed herein.

IV.D.12. HLA-A3 motif

The HLA-A3 motif is characterized by the presence in peptide ligands of L, M, V, I, S, A, T, F, C, G, or D as a primary anchor residue at position 2, and the presence of K, sY, R, H, F, or A as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.*, DiBrino *et al.*, *Proc. Natl. Acad. Sci USA* 90:1508, 1993; and Kubo *et al.*, *J. Immunol.* 152:3913-3924, 1994). Peptide binding to HLA-A3 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A3 motif are set forth in Table XVI. Those peptide epitopes that also comprise the A3 supermotif are also listed in Table IX. The A3 supermotif primary anchor residues comprise a subset of the A3- and A11-allele specific motif primary anchor residues.

IV.D.13. HLA-A11 motif

The HLA-A11 motif is characterized by the presence in peptide ligands of V, T, M, L, I, S, A, G, N, C, D, or F as a primary anchor residue in position 2, and K, R, Y, or H as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.*, Zhang *et al.*, *Proc. Natl. Acad. Sci USA* 90:2217-2221, 1993; and Kubo *et al.*, *J. Immunol.* 152:3913-3924, 1994). Peptide binding to HLA-A11 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A11 motif are set forth in Table XVII; peptide epitopes comprising the A3 allele-specific motif are also present in this Table because of the extensive overlap between the A3 and A11 motif primary anchor specificities. Further, those peptide epitopes that comprise the A3 supermotif are also listed in Table IX.

IV.D.14. HLA-A24 motif

The HLA-A24 motif is characterized by the presence in peptide ligands of Y, F, W, or M as a primary anchor residue in position 2, and F, L, I, or W as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.*, *Kondo et al.*, *J. Immunol.* 155:4307-4312, 1995; and Kubo *et al.*, *J. Immunol.* 152:3913-3924, 1994). Peptide binding to HLA-A24 molecules can be modulated by substitutions at primary and/or secondary anchor positions; preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A24 motif are set forth in Table XVIII. These epitopes are also listed in Table X, which sets forth HLA-A24-supermotif-bearing peptide epitopes, as the

primary anchor residues characterizing the A24 allele-specific motif comprise a subset of the A24 supermotif primary anchor residues.

Motifs Indicative of Class II HTL Inducing Peptide Epitopes

The primary and secondary anchor residues of the HLA class II peptide epitope supermotifs and motifs delineated below are summarized in Table III.

IV.D.15. HLA DR-1-4-7 supermotif

Motifs have also been identified for peptides that bind to three common HLA class II allele-specific HLA molecules: HLA DRB1*0401, DRB1*0101, and DRB1*0701 (*see, e.g.*, the review by Southwood *et al. J. Immunology* 160:3363-3373,1998). Collectively, the common residues from these motifs delineate the HLA DR-1-4-7 supermotif. Peptides that bind to these DR molecules carry a supermotif characterized by a large aromatic or hydrophobic residue (Y, F, W, L, I, V, or M) as a primary anchor residue in position 1, and a small, non-charged residue (S, T, C, A, P, V, I, L, or M) as a primary anchor residue in position 6 of a 9-mer core region. Allele-specific secondary effects and secondary anchors for each of these HLA types have also been identified (Southwood *et al., supra*). These are set forth in Table III. Peptide binding to HLA- DRB1*0401, DRB1*0101, and/or DRB1*0701 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Potential epitope 9-mer core regions comprising the DR-1-4-7 supermotif, wherein position 1 of the supermotif is at position 1 of the nine-residue core, are set forth in Table XIX. Respective exemplary peptide epitopes of 15 amino acid residues in length, each of which comprise a nine residue core, are also shown, along with cross-reactive binding data for the exemplary 15-residue peptides.

IV.D.16. HLA DR3 motifs

Two alternative motifs (*i.e.*, submotifs) characterize peptide epitopes that bind to HLA-DR3 molecules (*see, e.g.*, Geluk *et al., J. Immunol.* 152:5742, 1994). In the first motif (submotif DR3a) a large, hydrophobic residue (L, I, V, M, F, or Y) is present in anchor position 1 of a 9-mer core, and D is present as an anchor at position 4, towards the carboxyl terminus of the epitope. As in other class II motifs, core position 1 may or may not occupy the peptide N-terminal position.

The alternative DR3 submotif provides for lack of the large, hydrophobic residue at anchor position 1, and/or lack of the negatively charged or amide-like anchor residue at position 4, by the presence of a positive charge at position 6 towards the carboxyl terminus of the epitope. Thus, for the alternative allele-specific DR3 motif (submotif DR3b): L, I, V, M, F, Y, A, or Y is present at anchor position 1; D, N, Q, E, S, or T is present at anchor position 4; and K, R, or H is present at anchor position 6. Peptide binding to HLA-DR3 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Potential peptide epitope 9-mer core regions corresponding to a nine residue sequence comprising the DR3a submotif (wherein position 1 of the motif is at position 1 of the nine residue core) are set forth in Table XXa. Respective exemplary peptide epitopes of 15 amino acid residues in length, each of which

comprise the nine residue core, are also shown in Table XXa along with binding data for the exemplary peptides.

Potential peptide epitope 9-mer core regions comprising the DR3b submotif and respective exemplary 15-mer peptides comprising the DR3 submotif-b epitope are set forth in Table XXb along with binding data for the exemplary peptides.

Each of the HLA class I or class II peptide epitopes set out in the Tables herein are deemed singly to be an inventive aspect of this application. Further, it is also an inventive aspect of this application that each peptide epitope may be used in combination with any other peptide epitope.

IV.E. Enhancing Population Coverage of the Vaccine

Vaccines that have broad population coverage are preferred because they are more commercially viable and generally applicable to the most people. Broad population coverage can be obtained using the peptides of the invention (and nucleic acid compositions that encode such peptides) through selecting peptide epitopes that bind to HLA alleles which, when considered in total, are present in most of the population. Table XXI lists the overall frequencies of the HLA class I supertypes in various ethnicities (Table XXIa) and the combined population coverage achieved by the A2-, A3-, and B7-supertypes (Table XXIb). The A2-, A3-, and B7 supertypes are each present on the average of over 40% in each of these five major ethnic groups. Coverage in excess of 80% is achieved with a combination of these supermotifs. These results suggest that effective and non-ethnically biased population coverage is achieved upon use of a limited number of cross-reactive peptides. Although the population coverage reached with these three main peptide specificities is high, coverage can be expanded to reach 95% population coverage and above, and more easily achieve truly multispecific responses upon use of additional supermotif or allele-specific motif bearing peptides.

The B44-, A1-, and A24-supertypes are each present, on average, in a range from 25% to 40% in these major ethnic populations (Table XXIa). While less prevalent overall, the B27-, B58-, and B62 supertypes are each present with a frequency >25% in at least one major ethnic group (Table XXIa). Table XXIb summarizes the estimated prevalence of combinations of HLA supertypes that have been identified in five major ethnic groups. The incremental coverage obtained by the inclusion of A1,- A24-, and B44- supertypes to the A2, A3, and B7 coverage and coverage obtained with all of the supertypes described herein, is shown.

The data presented herein, together with the previous definition of the A2-, A3-, and B7- supertypes, indicates that all antigens, with the possible exception of A29, B8, and B46, can be classified into a total of nine HLA supertypes. By including epitopes from the six most frequent supertypes, an average population coverage of 99% is obtained for five major ethnic groups.

IV.F. Immune Response-Stimulating Peptide Analogs

In general, CTL and HTL responses are not directed against all possible epitopes. Rather, they are restricted to a few "immunodominant" determinants (Zinkernagel, *et al., Adv. Immunol.* 27:5159, 1979; Bennink, *et al., J. Exp. Med.* 168:19351939, 1988; Rawle, *et al., J. Immunol.* 146:3977-3984, 1991). It has been recognized that immunodominance (Benacerraf, *et al., Science* 175:273-279, 1972) could be explained

by either the ability of a given epitope to selectively bind a particular HLA protein (determinant selection theory) (Vitiello, *et al., J. Immunol.* 131:1635, 1983); Rosenthal, *et al., Nature* 267:156-158, 1977), or to be selectively recognized by the existing TCR (T cell receptor) specificities (repertoire theory) (Klein, J., IMMUNOLOGY, THE SCIENCE OF SELF/NONSELF DISCRIMINATION, John Wiley & Sons, New York, pp. 270-310, 1982). It has been demonstrated that additional factors, mostly linked to processing events, can also play a key role in dictating, beyond strict immunogenicity, which of the many potential determinants will be presented as immunodominant (Sercarz, *et al., Annu. Rev. Immunol.* 11:729-766, 1993).

Because tissue specific and developmental TAAs are expressed on normal tissue at least at some point in time or location within the body, it may be expected that T cells to them, particularly dominant epitopes, are eliminated during immunological surveillance and that tolerance is induced. However, CTL responses to tumor epitopes in both normal donors and cancer patient has been detected, which may indicate that tolerance is incomplete (*see, e.g.*, Kawashima *et al.*, Hum. Immunol. 59:1, 1998; Tsang, *J. Natl. Cancer Inst.* 87:82-90, 1995; Rongcun *et al., J. Immunol.* 163:1037, 1999). Thus, immune tolerance does not completely eliminate or inactivate CTL precursors capable of recognizing high affinity HLA class I binding peptides.

An additional strategy to overcome tolerance is to use analog peptides. Without intending to be bound by theory, it is believed that because T cells to dominant epitopes may have been clonally deleted, selecting subdominant epitopes may allow existing T cells to be recruited, which will then lead to a therapeutic or prophylactic response. However, the binding of HLA molecules to subdominant epitopes is often less vigorous than to dominant ones. Accordingly, there is a need to be able to modulate the binding affinity of particular immunogenic epitopes for one or more HLA molecules, and thereby to modulate the immune response elicited by the peptide, for example to prepare analog peptides which elicit a more vigorous response.

Although peptides with suitable cross-reactivity among all alleles of a superfamily are identified by the screening procedures described above, cross-reactivity is not always as complete as possible, and in certain cases procedures to increase cross-reactivity of peptides can be useful; moreover, such procedures can also be used to modify other properties of the peptides such as binding affinity or peptide stability. Having established the general rules that govern cross-reactivity of peptides for HLA alleles within a given motif or supermotif, modification (*i.e.*, analoging) of the structure of peptides of particular interest in order to achieve broader (or otherwise modified) HLA binding capacity can be performed. More specifically, peptides which exhibit the broadest cross-reactivity patterns, can be produced in accordance with the teachings herein. The present concepts related to analog generation are set forth in greater detail in co-pending U.S.S.N. 09/226,775 filed 1/6/99.

In brief, the strategy employed utilizes the motifs or supermotifs which correlate with binding to certain HLA molecules. The motifs or supermotifs are defined by having primary anchors, and in many cases secondary anchors. Analog peptides can be created by substituting amino acid residues at primary anchor, secondary anchor, or at primary and secondary anchor positions. Generally, analogs are made for peptides that already bear a motif or supermotif. Preferred secondary anchor residues of supermotifs and motifs that have been defined for HLA class I and class II binding peptides are shown in Tables II and III, respectively.

For a number of the motifs or supermotifs in accordance with the invention, residues are defined which are deleterious to binding to allele-specific HLA molecules or members of HLA supertypes that bind the respective motif or supermotif (Tables II and III). Accordingly, removal of such residues that are detrimental to binding can be performed in accordance with the present invention. For example, in the case of the A3 supertype, when all peptides that have such deleterious residues are removed from the population of peptides used in the analysis, the incidence of cross-reactivity increased from 22% to 37% (*see, e.g.*, Sidney, J. *et al.*, *Hu. Immunol.* 45:79, 1996). Thus, one strategy to improve the cross-reactivity of peptides within a given supermotif is simply to delete one or more of the deleterious residues present within a peptide and substitute a small "neutral" residue such as Ala (that may not influence T cell recognition of the peptide). An enhanced likelihood of cross-reactivity is expected if, together with elimination of detrimental residues within a peptide, "preferred" residues associated with high affinity binding to an allele-specific HLA molecule or to multiple HLA molecules within a superfamily are inserted.

To ensure that an analog peptide, when used as a vaccine, actually elicits a CTL response to the native epitope *in vivo* (or, in the case of class II epitopes, elicits helper T cells that cross-react with the wild type peptides), the analog peptide may be used to immunize T cells *in vitro* from individuals of the appropriate HLA allele. Thereafter, the immunized cells' capacity to induce lysis of wild type peptide sensitized target cells is evaluated. It will be desirable to use as antigen presenting cells, cells that have been either infected, or transfected with the appropriate genes, or, in the case of class II epitopes only, cells that have been pulsed with whole protein antigens, to establish whether endogenously produced antigen is also recognized by the relevant T cells.

Another embodiment of the invention is to create analogs of weak binding peptides, to thereby ensure adequate numbers of cross-reactive cellular binders. Class I binding peptides exhibiting binding affinities of 500-5000 nM, and carrying an acceptable but suboptimal primary anchor residue at one or both positions can be "fixed" by substituting preferred anchor residues in accordance with the respective supertype. The analog peptides can then be tested for crossbinding activity.

Another embodiment for generating effective peptide analogs involves the substitution of residues that have an adverse impact on peptide stability or solubility in, *e.g.*, a liquid environment. This substitution may occur at any position of the peptide epitope. For example, a cysteine can be substituted out in favor of α-amino butyric acid ("B" in the single letter abbreviations for peptide sequences listed herein). Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally so as to reduce binding capacity. Substituting α-amino butyric acid for cysteine not only alleviates this problem, but actually improves binding and crossbinding capability in certain instances (*see, e.g.*, the review by Sette *et al.*, In: Persistent Viral Infections, Eds. R. Ahmed and I. Chen, John Wiley & Sons, England, 1999).

Representative analog peptides are set forth in Tables XXII-XXVI. The Table indicates the length and sequence of the analog peptide as well as the motif or supermotif, if appropriate. The "source" column indicates the residues substituted at the indicated position numbers for the respective analog.

IV.G. Computer Screening of Protein Sequences from Disease-Related Antigens for Supermotif- or Motif-Bearing Peptides

In order to identify supermotif- or motif-bearing epitopes in a target antigen, a native protein sequence, *e.g.*, a tumor-associated antigen, or sequences from an infectious organism, or a donor tissue for transplantation, is screened using a means for computing, such as an intellectual calculation or a computer, to determine the presence of a supermotif or motif within the sequence. The information obtained from the analysis of native peptide can be used directly to evaluate the status of the native peptide or may be utilized subsequently to generate the peptide epitope.

Computer programs that allow the rapid screening of protein sequences for the occurrence of the subject supermotifs or motifs are encompassed by the present invention; as are programs that permit the generation of analog peptides. These programs are implemented to analyze any identified amino acid sequence or operate on an unknown sequence and simultaneously determine the sequence and identify motif-bearing epitopes thereof; analogs can be simultaneously determined as well. Generally, the identified sequences will be from a pathogenic organism or a tumor-associated peptide. For example, the target TAA molecules include, without limitation, CEA, MAGE, p53 and HER2/neu.

It is important that the selection criteria utilized for prediction of peptide binding are as accurate as possible, to correlate most efficiently with actual binding. Prediction of peptides that bind, for example, to HLA-A*0201, on the basis of the presence of the appropriate primary anchors, is positive at about a 30% rate (*see, e.g.*, Ruppert, J. *et al. Cell* 74:929, 1993). However, by extensively analyzing peptide-HLA binding data disclosed herein, data in related patent applications, and data in the art, the present inventors have developed a number of allele-specific polynomial algorithms that dramatically increase the predictive value over identification on the basis of the presence of primary anchor residues alone. These algorithms take into account not only the presence or absence of primary anchors, but also consider the positive or deleterious presence of secondary anchor residues (to account for the impact of different amino acids at different positions). The algorithms are essentially based on the premise that the overall affinity (or ΔG) of peptide-HLA interactions can be approximated as a linear polynomial function of the type:

$$\Delta G = a_{1i} \times a_{2i} \times a_{3i} \ldots \times a_{ni}$$

where a_{ji} is a coefficient that represents the effect of the presence of a given amino acid (j) at a given position (i) along the sequence of a peptide of n amino acids. An important assumption of this method is that the effects at each position are essentially independent of each other. This assumption is justified by studies that demonstrated that peptides are bound to HLA molecules and recognized by T cells in essentially an extended conformation. Derivation of specific algorithm coefficients has been described, for example, in Gulukota, K. *et al., J. Mol. Biol.* 267:1258, 1997.

Additional methods to identify preferred peptide sequences, which also make use of specific motifs, include the use of neural networks and molecular modeling programs (*see, e.g.*, Milik *et al., Nature Biotechnology* 16:753, 1998; Altuvia *et al., Hum. Immunol.* 58:1, 1997; Altuvia *et al, J. Mol. Biol.* 249:244, 1995; Buus, S. *Curr. Opin. Immunol.* 11:209-213, 1999; Brusic, V. *et al., Bioinformatics* 14:121-130, 1998; Parker *et al., J. Immunol.* 152:163, 1993; Meister *et al., Vaccine* 13:581, 1995; Hammer *et al., J. Exp. Med.* 180:2353, 1994; Sturniolo *et al., Nature Biotechnol.* 17:555 1999).

For example, it has been shown that in sets of A*0201 motif-bearing peptides containing at least one preferred secondary anchor residue while avoiding the presence of any deleterious secondary anchor residues, 69% of the peptides will bind A*0201 with an IC_{50} less than 500 nM (Ruppert, J. *et al. Cell* 74:929, 1993). These algorithms are also flexible in that cut-off scores may be adjusted to select sets of peptides with greater or lower predicted binding properties, as desired.

In utilizing computer screening to identify peptide epitopes, a protein sequence or translated sequence may be analyzed using software developed to search for motifs, for example the "FINDPATTERNS' program (Devereux, *et al. Nucl. Acids Res.* 12:387-395, 1984) or MotifSearch 1.4 software program (D. Brown, San Diego, CA) to identify potential peptide sequences containing appropriate HLA binding motifs. The identified peptides can be scored using customized polynomial algorithms to predict their capacity to bind specific HLA class I or class II alleles. As appreciated by one of ordinary skill in the art, a large array of computer programming software and hardware options are available in the relevant art which can be employed to implement the motifs of the invention in order to evaluate (*e.g.*, without limitation, to identify epitopes, identify epitope concentration per peptide length, or to generate analogs) known or unknown peptide sequences.

In accordance with the procedures described above, HER2/neu peptide epitopes and analogs thereof that are able to bind HLA supertype groups or allele-specific HLA molecules have been identified (Tables VII-XX; Table XXII-XXXI).

IV.H. Preparation of Peptide Epitopes

Peptides in accordance with the invention can be prepared synthetically, by recombinant DNA technology or chemical synthesis, or from natural sources such as native tumors or pathogenic organisms. Peptide epitopes may be synthesized individually or as polyepitopic peptides. Although the peptide will preferably be substantially free of other naturally occurring host cell proteins and fragments thereof, in some embodiments the peptides may be synthetically conjugated to native fragments or particles.

The peptides in accordance with the invention can be a variety of lengths, and either in their neutral (uncharged) forms or in forms which are salts. The peptides in accordance with the invention are either free of modifications such as glycosylation, side chain oxidation, or phosphorylation; or they contain these modifications, subject to the condition that modifications do not destroy the biological activity of the peptides as described herein.

When possible, it may be desirable to optimize HLA class I binding epitopes of the invention, such as can be used in a polyepitopic construct, to a length of about 8 to about 13 amino acid residues, often 8 to 11, preferably 9 to 10. HLA class II binding peptide epitopes of the invention may be optimized to a length of about 6 to about 30 amino acids in length, preferably to between about 13 and about 20 residues. Preferably, the peptide epitopes are commensurate in size with endogenously processed pathogen-derived peptides or tumor cell peptides that are bound to the relevant HLA molecules, however, the identification and preparation of peptides that comprise epitopes of the invention can also be carried out using the techniques described herein.

In alternative embodiments, epitopes of the invention can be linked as a polyepitopic peptide, or as a minigene that encodes a polyepitopic peptide.

In another embodiment, it is preferred to identify native peptide regions that contain a high concentration of class I and/or class II epitopes. Such a sequence is generally selected on the basis that it contains the greatest number of epitopes per amino acid length. It is to be appreciated that epitopes can be present in a nested or overlapping manner, *e.g.* a 10 amino acid long peptide could contain two 9 amino acid long epitopes and one 10 amino acid long epitope; upon intracellular processing, each epitope can be exposed and bound by an HLA molecule upon administration of such a peptide. This larger, preferably multi-epitopic, peptide can be generated synthetically, recombinantly, or via cleavage from the native source.

The peptides of the invention can be prepared in a wide variety of ways. For the preferred relatively short size, the peptides can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. (*See*, for example, Stewart & Young, SOLID PHASE PEPTIDE SYNTHESIS, 2D. ED., Pierce Chemical Co., 1984). Further, individual peptide epitopes can be joined using chemical ligation to produce larger peptides that are still within the bounds of the invention.

Alternatively, recombinant DNA technology can be employed wherein a nucleotide sequence which encodes an immunogenic peptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression. These procedures are generally known in the art, as described generally in Sambrook *et al.*, MOLECULAR CLONING, A LABORATORY MANUAL, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989). Thus, recombinant polypeptides which comprise one or more peptide sequences of the invention can be used to present the appropriate T cell epitope.

The nucleotide coding sequence for peptide epitopes of the preferred lengths contemplated herein can be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci, *et al., J. Am. Chem. Soc.* 103:3185 (1981). Peptide analogs can be made simply by substituting the appropriate and desired nucleic acid base(s) for those that encode the native peptide sequence; exemplary nucleic acid substitutions are those that encode an amino acid defined by the motifs/supermotifs herein. The coding sequence can then be provided with appropriate linkers and ligated into expression vectors commonly available in the art, and the vectors used to transform suitable hosts to produce the desired fusion protein. A number of such vectors and suitable host systems are now available. For expression of the fusion proteins, the coding sequence will be provided with operably linked start and stop codons, promoter and terminator regions and usually a replication system to provide an expression vector for expression in the desired cellular host. For example, promoter sequences compatible with bacterial hosts are provided in plasmids containing convenient restriction sites for insertion of the desired coding sequence. The resulting expression vectors are transformed into suitable bacterial hosts. Of course, yeast, insect or mammalian cell hosts may also be used, employing suitable vectors and control sequences.

IV.I. Assays to Detect T-Cell Responses

Once HLA binding peptides are identified, they can be tested for the ability to elicit a T-cell response. The preparation and evaluation of motif-bearing peptides are described in PCT publications WO 94/20127 and WO 94/03205. Briefly, peptides comprising epitopes from a particular antigen are

synthesized and tested for their ability to bind to the appropriate HLA proteins. These assays may involve evaluating the binding of a peptide of the invention to purified HLA class I molecules in relation to the binding of a radioiodinated reference peptide. Alternatively, cells expressing empty class I molecules (*i.e.* lacking peptide therein) may be evaluated for peptide binding by immunofluorescent staining and flow microfluorimetry. Other assays that may be used to evaluate peptide binding include peptide-dependent class I assembly assays and/or the inhibition of CTL recognition by peptide competition. Those peptides that bind to the class I molecule, typically with an affinity of 500 nM or less, are further evaluated for their ability to serve as targets for CTLs derived from infected or immunized individuals, as well as for their capacity to induce primary *in vitro* or *in vivo* CTL responses that can give rise to CTL populations capable of reacting with selected target cells associated with a disease. Corresponding assays are used for evaluation of HLA class II binding peptides. HLA class II motif-bearing peptides that are shown to bind, typically at an affinity of 1000 nM or less, are further evaluated for the ability to stimulate HTL responses.

Conventional assays utilized to detect T cell responses include proliferation assays, lymphokine secretion assays, direct cytotoxicity assays, and limiting dilution assays. For example, antigen-presenting cells that have been incubated with a peptide can be assayed for the ability to induce CTL responses in responder cell populations. Antigen-presenting cells can be normal cells such as peripheral blood mononuclear cells or dendritic cells. Alternatively, mutant non-human mammalian cell lines that are deficient in their ability to load class I molecules with internally processed peptides and that have been transfected with the appropriate human class I gene, may be used to test for the capacity of the peptide to induce *in vitro* primary CTL responses.

Peripheral blood mononuclear cells (PBMCs) may be used as the responder cell source of CTL precursors. The appropriate antigen-presenting cells are incubated with peptide, after which the peptide-loaded antigen-presenting cells are then incubated with the responder cell population under optimized culture conditions. Positive CTL activation can be determined by assaying the culture for the presence of CTLs that kill radio-labeled target cells, both specific peptide-pulsed targets as well as target cells expressing endogenously processed forms of the antigen from which the peptide sequence was derived.

More recently, a method has been devised which allows direct quantification of antigen-specific T cells by staining with Fluorescein-labelled HLA tetrameric complexes (Altman, J. D. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:10330, 1993; Altman, J. D. *et al.*, *Science* 274:94, 1996). Other relatively recent technical developments include staining for intracellular lymphokines, and interferon-γ release assays or ELISPOT assays. Tetramer staining, intracellular lymphokine staining and ELISPOT assays all appear to be at least 10-fold more sensitive than more conventional assays (Lalvani, A. *et al.*, *J. Exp. Med.* 186:859, 1997; Dunbar, P. R. *et al.*, *Curr. Biol.* 8:413, 1998; Murali-Krishna, K. *et al.*, *Immunity* 8:177, 1998).

HTL activation may also be assessed using such techniques known to those in the art such as T cell proliferation and secretion of lymphokines, *e.g.* IL-2 (*see, e.g.* Alexander *et al.*, Immunity 1:751-761, 1994).

Alternatively, immunization of HLA transgenic mice can be used to determine immunogenicity of peptide epitopes. Several transgenic mouse models including mice with human A2.1, A11 (which can additionally be used to analyze HLA-A3 epitopes), and B7 alleles have been characterized and others (*e.g.*, transgenic mice for HLA-A1 and A24) are being developed. HLA-DR1 and HLA-DR3 mouse models have

also been developed. Additional transgenic mouse models with other HLA alleles may be generated as necessary. Mice may be immunized with peptides emulsified in Incomplete Freund's Adjuvant and the resulting T cells tested for their capacity to recognize peptide-pulsed target cells and target cells transfected with appropriate genes. CTL responses may be analyzed using cytotoxicity assays described above. Similarly, HTL responses may be analyzed using such assays as T cell proliferation or secretion of lymphokines.

IV.J. Use of Peptide Epitopes as Diagnostic Agents and for Evaluating Immune Responses

In one embodiment of the invention, HLA class I and class II binding peptides as described herein are used as reagents to evaluate an immune response. The immune response to be evaluated is induced by using as an immunogen any agent that may result in the production of antigen-specific CTLs or HTLs that recognize and bind to the peptide epitope(s) to be employed as the reagent. The peptide reagent need not be used as the immunogen. Assay systems that are used for such an analysis include relatively recent technical developments such as tetramers, staining for intracellular lymphokines and interferon release assays, or ELISPOT assays.

For example, peptides of the invention are used in tetramer staining assays to assess peripheral blood mononuclear cells for the presence of antigen-specific CTLs following exposure to a tumor cell antigen or an immunogen. The HLA-tetrameric complex is used to directly visualize antigen-specific CTLs (*see, e.g.*, Ogg *et al.*, *Science* 279:2103-2106, 1998; and Altman *et al.*, *Science* 174:94-96, 1996) and determine the frequency of the antigen-specific CTL population in a sample of peripheral blood mononuclear cells. A tetramer reagent using a peptide of the invention is generated as follows: A peptide that binds to an HLA molecule is refolded in the presence of the corresponding HLA heavy chain and β_2-microglobulin to generate a trimolecular complex. The complex is biotinylated at the carboxyl terminal end of the heavy chain at a site that was previously engineered into the protein. Tetramer formation is then induced by the addition of streptavidin. By means of fluorescently labeled streptavidin, the tetramer can be used to stain antigen-specific cells. The cells can then be identified, for example, by flow cytometry. Such an analysis may be used for diagnostic or prognostic purposes. Cells identified by the procedure can also be used for therapeutic purposes.

Peptides of the invention are also used as reagents to evaluate immune recall responses (*see, e.g.*, Bertoni *et al.*, *J. Clin. Invest.* 100:503-513, 1997 and Penna *et al.*, *J. Exp. Med.* 174:1565-1570, 1991). For example, patient PBMC samples from individuals with cancer are analyzed for the presence of antigen-specific CTLs or HTLs using specific peptides. A blood sample containing mononuclear cells can be evaluated by cultivating the PBMCs and stimulating the cells with a peptide of the invention. After an appropriate cultivation period, the expanded cell population can be analyzed, for example, for CTL or for HTL activity.

The peptides are also used as reagents to evaluate the efficacy of a vaccine. PBMCs obtained from a patient vaccinated with an immunogen are analyzed using, for example, either of the methods described above. The patient is HLA typed, and peptide epitope reagents that recognize the allele-specific molecules present in that patient are selected for the analysis. The immunogenicity of the vaccine is indicated by the presence of epitope-specific CTLs and/or HTLs in the PBMC sample.

The peptides of the invention are also used to make antibodies, using techniques well known in the art (see, *e.g. CURRENT PROTOCOLS IN IMMUNOLOGY*, Wiley/Greene, NY; and *Antibodies A Laboratory Manual*, Harlow and Lane, Cold Spring Harbor Laboratory Press, 1989), which may be useful as reagents to diagnose or monitor cancer. Such antibodies include those that recognize a peptide in the context of an HLA molecule, *i.e.*, antibodies that bind to a peptide-MHC complex.

IV.K. Vaccine Compositions

Vaccines and methods of preparing vaccines that contain an immunogenically effective amount of one or more peptides as described herein are further embodiments of the invention. Once appropriately immunogenic epitopes have been defined, they can be sorted and delivered by various means, herein referred to as "vaccine" compositions. Such vaccine compositions can include, for example, lipopeptides (*e.g.*,Vitiello, A. *et al.*, *J. Clin. Invest.* 95:341, 1995), peptide compositions encapsulated in poly(DL-lactide-co-glycolide) ("PLG") microspheres (*see, e.g.*, Eldridge, *et al.*, *Molec. Immunol.* 28:287-294, 1991: Alonso *et al.*, *Vaccine* 12:299-306, 1994; Jones *et al.*, *Vaccine* 13:675-681, 1995), peptide compositions contained in immune stimulating complexes (ISCOMS) (*see, e.g.*, Takahashi *et al.*, *Nature* 344:873-875, 1990; Hu *et al.*, *Clin Exp Immunol.* 113:235-243, 1998), multiple antigen peptide systems (MAPs) (*see e.g.*, Tam, J. P., *Proc. Natl. Acad. Sci. U.S.A.* 85:5409-5413, 1988; Tam, J.P., *J. Immunol. Methods* 196:17-32, 1996), peptides formulated as multivalent peptides; peptides for use in ballistic delivery systems, typically crystallized peptides, viral delivery vectors (Perkus, M. E. *et al.*, In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 379, 1996; Chakrabarti, S. *et al.*, *Nature* 320:535, 1986; Hu, S. L. *et al.*, *Nature* 320:537, 1986; Kieny, M.-P. *et al.*, *AIDS Bio/Technology* 4:790, 1986; Top, F. H. *et al.*, *J. Infect. Dis.* 124:148, 1971; Chanda, P. K. *et al.*, *Virology* 175:535, 1990), particles of viral or synthetic origin (*e.g.*, Kofler, N. *et al.*, *J. Immunol. Methods.* 192:25, 1996; Eldridge, J. H. *et al.*, *Sem. Hematol.* 30:16, 1993; Falo, L. D., Jr. *et al.*, *Nature Med.* 7:649, 1995), adjuvants (Warren, H. S., Vogel, F. R., and Chedid, L. A. *Annu. Rev. Immunol.* 4:369, 1986; Gupta, R. K. *et al.*, *Vaccine* 11:293, 1993), liposomes (Reddy, R. *et al.*, *J. Immunol.* 148:1585, 1992; Rock, K. L., *Immunol. Today* 17:131, 1996), or, naked or particle absorbed cDNA (Ulmer, J. B. *et al.*, *Science* 259:1745, 1993; Robinson, H. L., Hunt, L. A., and Webster, R. G., *Vaccine* 11:957, 1993; Shiver, J. W. *et al.*, In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 423, 1996; Cease, K. B., and Berzofsky, J. A., *Annu. Rev. Immunol.* 12:923, 1994 and Eldridge, J. H. *et al.*, *Sem. Hematol.* 30:16, 1993). Toxin-targeted delivery technologies, also known as receptor mediated targeting, such as those of Avant Immunotherapeutics, Inc. (Needham, Massachusetts) may also be used.

Vaccines of the invention include nucleic acid-mediated modalities. DNA or RNA encoding one or more of the peptides of the invention can also be administered to a patient. This approach is described, for instance, in Wolff *et. al.*, *Science* 247:1465 (1990) as well as U.S. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; WO 98/04720; and in more detail below. Examples of DNA-based delivery technologies include "naked DNA", facilitated (bupivicaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") or pressure-mediated delivery (*see, e.g.*, U.S. Patent No. 5,922,687).

For therapeutic or prophylactic immunization purposes, the peptides of the invention can also be expressed by viral or bacterial vectors. Examples of expression vectors include attenuated viral hosts, such

as vaccinia or fowlpox. As an example of this approach, vaccinia virus is used as a vector to express nucleotide sequences that encode the peptides of the invention. Upon introduction into a host bearing a tumor, the recombinant vaccinia virus expresses the immunogenic peptide, and thereby elicits a host CTL and/or HTL response. Vaccinia vectors and methods useful in immunization protocols are described in, *e.g.*, U.S. Patent No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover *et al.*, *Nature* 351:456-460 (1991). A wide variety of other vectors useful for therapeutic administration or immunization of the peptides of the invention, *e.g.* adeno and adeno-associated virus vectors, retroviral vectors, *Salmonella typhi* vectors, detoxified anthrax toxin vectors, and the like, will be apparent to those skilled in the art from the description herein.

Furthermore, vaccines in accordance with the invention encompass compositions of one or more of the claimed peptides. A peptide can be present in a vaccine individually. Alternatively, the peptide can exist as a homopolymer comprising multiple copies of the same peptide, or as a heteropolymer of various peptides. Polymers have the advantage of increased immunological reaction and, where different peptide epitopes are used to make up the polymer, the additional ability to induce antibodies and/or CTLs that react with different antigenic determinants of the pathogenic organism or tumor-related peptide targeted for an immune response. The composition can be a naturally occurring region of an antigen or can be prepared, *e.g.*, recombinantly or by chemical synthesis.

Carriers that can be used with vaccines of the invention are well known in the art, and include, *e.g.*, thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly L-lysine, poly L-glutamic acid, influenza, hepatitis B virus core protein, and the like. The vaccines can contain a physiologically tolerable (*i.e.*, acceptable) diluent such as water, or saline, preferably phosphate buffered saline. The vaccines also typically include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are examples of materials well known in the art. Additionally, as disclosed herein, CTL responses can be primed by conjugating peptides of the invention to lipids, such as tripalmitoyl-S-glycerylcysteinlyseryl- serine (P_3CSS).

Upon immunization with a peptide composition in accordance with the invention, via injection, aerosol, oral, transdermal, transmucosal, intrapleural, intrathecal, or other suitable routes, the immune system of the host responds to the vaccine by producing large amounts of CTLs and/or HTLs specific for the desired antigen. Consequently, the host becomes at least partially immune to later infection, or at least partially resistant to developing an ongoing chronic infection, or derives at least some therapeutic benefit when the antigen was tumor-associated.

In some embodiments, it may be desirable to combine the class I peptide components with components that induce or facilitate neutralizing antibody and or helper T cell responses to the target antigen of interest. A preferred embodiment of such a composition comprises class I and class II epitopes in accordance with the invention. An alternative embodiment of such a composition comprises a class I and/or class II epitope in accordance with the invention, along with a cross-binding HLA class II epitope such as PADRE™ (Epimmune, San Diego, CA) molecule (described, for example, in U.S. Patent Number 5,736,142).

A vaccine of the invention can also include antigen-presenting cells (APC), such as dendritic cells (DC), as a vehicle to present peptides of the invention. Vaccine compositions can be created *in vitro*,

following dendritic cell mobilization and harvesting, whereby loading of dendritic cells occurs *in vitro*. For example, dendritic cells are transfected, *e.g.*, with a minigene in accordance with the invention, or are pulsed with peptides. The dendritic cell can then be administered to a patient to elicit immune responses *in vivo*.

Vaccine compositions, either DNA- or peptide-based, can also be administered *in vivo* in combination with dendritic cell mobilization whereby loading of dendritic cells occurs *in vivo*.

Antigenic peptides are used to elicit a CTL and/or HTL response *ex vivo*, as well. The resulting CTL or HTL cells, can be used to treat tumors in patients that do not respond to other conventional forms of therapy, or will not respond to a therapeutic vaccine peptide or nucleic acid in accordance with the invention. *Ex vivo* CTL or HTL responses to a particular tumor-associated antigen are induced by incubating in tissue culture the patient's, or genetically compatible, CTL or HTL precursor cells together with a source of antigen-presenting cells, such as dendritic cells, and the appropriate immunogenic peptide. After an appropriate incubation time (typically about 7-28 days), in which the precursor cells are activated and expanded into effector cells, the cells are infused back into the patient, where they will destroy (CTL) or facilitate destruction (HTL) of their specific target cell (an infected cell or a tumor cell). Transfected dendritic cells may also be used as antigen presenting cells.

The vaccine compositions of the invention can also be used in combination with other treatments used for cancer, including use in combination with immune adjuvants such as IL-2, IL-12, GM-CSF, and the like.

Preferably, the following principles are utilized when selecting an array of epitopes for inclusion in a polyepitopic composition for use in a vaccine, or for selecting discrete epitopes to be included in a vaccine and/or to be encoded by nucleic acids such as a minigene. Examples of epitopes that can be utilized in a vaccine to treat or prevent cancer are provided in Tables XXIII-XXVII and XXXI. It is preferred that each of the following principles are balanced in order to make the selection. The multiple epitopes to be incorporated in a given vaccine composition can be, but need not be, contiguous in sequence in the native antigen from which the epitopes are derived.

1.) Epitopes are selected which, upon administration, mimic immune responses that have been observed to be correlated with tumor clearance. For HLA Class I this includes 3-4 epitopes that come from at least one TAA. For HLA Class II a similar rationale is employed; again 3-4 epitopes are selected from at least one TAA (*see e.g.*, Rosenberg *et al.*, *Science* 278:1447-1450). Epitopes from one TAA may be used in combination with epitopes from one or more additional TAAs to produce a vaccine that targets tumors with varying expression patterns of frequently-expressed TAAs as described, *e.g.*, in Example 15.

2.) Epitopes are selected that have the requisite binding affinity established to be correlated with immunogenicity: for HLA Class I an IC_{50} of 500 nM or less, or for Class II an IC_{50} of 1000 nM or less.

3.) Sufficient supermotif bearing-peptides, or a sufficient array of allele-specific motif-bearing peptides, are selected to give broad population coverage. For example, it is preferable to have at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art, can be employed to assess the breadth, or redundancy of, population coverage.

4.) When selecting epitopes from cancer-related antigens it is often useful to select analogs because the patient may have developed tolerance to the native epitope. When selecting epitopes for infectious disease-related antigens it is preferable to select either native or analoged epitopes.

5.) Of particular relevance are epitopes referred to as "nested epitopes." Nested epitopes occur where at least two epitopes overlap in a given peptide sequence. A nested peptide sequence can comprise both HLA class I and HLA class II epitopes. When providing nested epitopes, a general objective is to provide the greatest number of epitopes per sequence. Thus, an aspect is to avoid providing a peptide that is any longer than the amino terminus of the amino terminal epitope and the carboxyl terminus of the carboxyl terminal epitope in the peptide. When providing a multi-epitopic sequence, such as a sequence comprising nested epitopes, it is generally important to screen the sequence in order to insure that it does not have pathological or other deleterious biological properties.

6.) If a polyepitopic protein is created, or when creating a minigene, an objective is to generate the smallest peptide that encompasses the epitopes of interest. This principle is similar, if not the same as that employed when selecting a peptide comprising nested epitopes. However, with an artificial polyepitopic peptide, the size minimization objective is balanced against the need to integrate any spacer sequences between epitopes in the polyepitopic protein. Spacer amino acid residues can, for example, be introduced to avoid junctional epitopes (an epitope recognized by the immune system, not present in the target antigen, and only created by the man-made juxtaposition of epitopes), or to facilitate cleavage between epitopes and thereby enhance epitope presentation. Junctional epitopes are generally to be avoided because the recipient may generate an immune response to that non-native epitope. Of particular concern is a junctional epitope that is a "dominant epitope." A dominant epitope may lead to such a zealous response that immune responses to other epitopes are diminished or suppressed.

IV.K.1. Minigene Vaccines

A number of different approaches are available which allow simultaneous delivery of multiple epitopes. Nucleic acids encoding the peptides of the invention are a particularly useful embodiment of the invention. Epitopes for inclusion in a minigene are preferably selected according to the guidelines set forth in the previous section. A preferred means of administering nucleic acids encoding the peptides of the invention uses minigene constructs encoding a peptide comprising one or multiple epitopes of the invention.

The use of multi-epitope minigenes is described below and in, *e.g.*, co-pending application U.S.S.N. 09/311,784; Ishioka *et al.*, *J. Immunol.* 162:3915-3925, 1999; An, L. and Whitton, J. L., *J. Virol.* 71:2292, 1997; Thomson, S. A. *et al.*, *J. Immunol.* 157:822, 1996; Whitton, J. L. *et al.*, *J. Virol.* 67:348, 1993; Hanke, R. *et al.*, *Vaccine* 16:426, 1998. For example, a multi-epitope DNA plasmid encoding supermotif- and/or motif-bearing HER2/neu epitopes derived from multiple regions of HER2/neu, a universal helper T epitope, *e.g.*, PADRE™ (or multiple HTL epitopes from HER2/neu), and an endoplasmic reticulum-translocating signal sequence can be engineered. A vaccine may also comprise epitopes, in addition to HER2/neu epitopes, that are derived from other TAAs.

The immunogenicity of a multi-epitopic minigene can be tested in transgenic mice to evaluate the magnitude of CTL induction responses against the epitopes tested. Further, the immunogenicity of DNA-

encoded epitopes *in vivo* can be correlated with the *in vitro* responses of specific CTL lines against target cells transfected with the DNA plasmid. Thus, these experiments can show that the minigene serves to both: 1.) generate a CTL response and 2.) that the induced CTLs recognized cells expressing the encoded epitopes.

For example, to create a DNA sequence encoding the selected epitopes (minigene) for expression in human cells, the amino acid sequences of the epitopes may be reverse translated. A human codon usage table can be used to guide the codon choice for each amino acid. These epitope-encoding DNA sequences may be directly adjoined, so that when translated, a continuous polypeptide sequence is created. To optimize expression and/or immunogenicity, additional elements can be incorporated into the minigene design. Examples of amino acid sequences that can be reverse translated and included in the minigene sequence include: HLA class I epitopes, HLA class II epitopes, a ubiquitination signal sequence, and/or an endoplasmic reticulum targeting signal. In addition, HLA presentation of CTL and HTL epitopes may be improved by including synthetic (*e.g.* poly-alanine) or naturally-occurring flanking sequences adjacent to the CTL or HTL epitopes; these larger peptides comprising the epitope(s) are within the scope of the invention.

The minigene sequence may be converted to DNA by assembling oligonucleotides that encode the plus and minus strands of the minigene. Overlapping oligonucleotides (30-100 bases long) may be synthesized, phosphorylated, purified and annealed under appropriate conditions using well known techniques. The ends of the oligonucleotides can be joined, for example, using T4 DNA ligase. This synthetic minigene, encoding the epitope polypeptide, can then be cloned into a desired expression vector.

Standard regulatory sequences well known to those of skill in the art are preferably included in the vector to ensure expression in the target cells. Several vector elements are desirable: a promoter with a down-stream cloning site for minigene insertion; a polyadenylation signal for efficient transcription termination; an *E. coli* origin of replication; and an *E. coli* selectable marker (*e.g.* ampicillin or kanamycin resistance). Numerous promoters can be used for this purpose, *e.g.*, the human cytomegalovirus (hCMV) promoter. See, *e.g.*, U.S. Patent Nos. 5,580,859 and 5,589,466 for other suitable promoter sequences.

Additional vector modifications may be desired to optimize minigene expression and immunogenicity. In some cases, introns are required for efficient gene expression, and one or more synthetic or naturally-occurring introns could be incorporated into the transcribed region of the minigene. The inclusion of mRNA stabilization sequences and sequences for replication in mammalian cells may also be considered for increasing minigene expression.

Once an expression vector is selected, the minigene is cloned into the polylinker region downstream of the promoter. This plasmid is transformed into an appropriate *E. coli* strain, and DNA is prepared using standard techniques. The orientation and DNA sequence of the minigene, as well as all other elements included in the vector, are confirmed using restriction mapping and DNA sequence analysis. Bacterial cells harboring the correct plasmid can be stored as a master cell bank and a working cell bank.

In addition, immunostimulatory sequences (ISSs or CpGs) appear to play a role in the immunogenicity of DNA vaccines. These sequences may be included in the vector, outside the minigene coding sequence, if desired to enhance immunogenicity.

In some embodiments, a bi-cistronic expression vector which allows production of both the minigene-encoded epitopes and a second protein (included to enhance or decrease immunogenicity) can be used. Examples of proteins or polypeptides that could beneficially enhance the immune response if co-expressed include cytokines (*e.g.*, IL-2, IL-12, GM-CSF), cytokine-inducing molecules (*e.g.*, LeIF), costimulatory molecules, or for HTL responses, pan-DR binding proteins (PADRE™, Epimmune, San Diego, CA). Helper (HTL) epitopes can be joined to intracellular targeting signals and expressed separately from expressed CTL epitopes; this allows direction of the HTL epitopes to a cell compartment different than that of the CTL epitopes. If required, this could facilitate more efficient entry of HTL epitopes into the HLA class II pathway, thereby improving HTL induction. In contrast to HTL or CTL induction, specifically decreasing the immune response by co-expression of immunosuppressive molecules (*e.g.* TGF-β) may be beneficial in certain diseases.

Therapeutic quantities of plasmid DNA can be produced for example, by fermentation in *E. coli*, followed by purification. Aliquots from the working cell bank are used to inoculate growth medium, and grown to saturation in shaker flasks or a bioreactor according to well known techniques. Plasmid DNA can be purified using standard bioseparation technologies such as solid phase anion-exchange resins supplied by QIAGEN, Inc. (Valencia, California). If required, supercoiled DNA can be isolated from the open circular and linear forms using gel electrophoresis or other methods.

Purified plasmid DNA can be prepared for injection using a variety of formulations. The simplest of these is reconstitution of lyophilized DNA in sterile phosphate-buffered saline (PBS). This approach, known as "naked DNA," is currently being used for intramuscular (IM) administration in clinical trials. To maximize the immunotherapeutic effects of minigene DNA vaccines, an alternative method for formulating purified plasmid DNA may be desirable. A variety of methods have been described, and new techniques may become available. Cationic lipids, glycolipids, and fusogenic liposomes can also be used in the formulation (see, *e.g.*, as described by WO 93/24640; Mannino & Gould-Fogerite, *BioTechniques* 6(7): 682 (1988); U.S. Pat No. 5,279,833; WO 91/06309; and Felgner, *et al., Proc. Nat'l Acad. Sci. USA* 84:7413 (1987). In addition, peptides and compounds referred to collectively as protective, interactive, non-condensing compounds (PINC) could also be complexed to purified plasmid DNA to influence variables such as stability, intramuscular dispersion, or trafficking to specific organs or cell types.

Target cell sensitization can be used as a functional assay for expression and HLA class I presentation of minigene-encoded CTL epitopes. For example, the plasmid DNA is introduced into a mammalian cell line that is suitable as a target for standard CTL chromium release assays. The transfection method used will be dependent on the final formulation. Electroporation can be used for "naked" DNA, whereas cationic lipids allow direct *in vitro* transfection. A plasmid expressing green fluorescent protein (GFP) can be co-transfected to allow enrichment of transfected cells using fluorescence activated cell sorting (FACS). These cells are then chromium-51 (^{51}Cr) labeled and used as target cells for epitope-specific CTL lines; cytolysis, detected by ^{51}Cr release, indicates both production of, and HLA presentation of, minigene-encoded CTL epitopes. Expression of HTL epitopes may be evaluated in an analogous manner using assays to assess HTL activity.

In vivo immunogenicity is a second approach for functional testing of minigene DNA formulations. Transgenic mice expressing appropriate human HLA proteins are immunized with the DNA

product. The dose and route of administration are formulation dependent (*e.g.*, IM for DNA in PBS, intraperitoneal (IP) for lipid-complexed DNA). Twenty-one days after immunization, splenocytes are harvested and restimulated for one week in the presence of peptides encoding each epitope being tested. Thereafter, for CTL effector cells, assays are conducted for cytolysis of peptide-loaded, ^{51}Cr-labeled target cells using standard techniques. Lysis of target cells that were sensitized by HLA loaded with peptide epitopes, corresponding to minigene-encoded epitopes, demonstrates DNA vaccine function for *in vivo* induction of CTLs. Immunogenicity of HTL epitopes is evaluated in transgenic mice in an analogous manner.

Alternatively, the nucleic acids can be administered using ballistic delivery as described, for instance, in U.S. Patent No. 5,204,253. Using this technique, particles comprised solely of DNA are administered. In a further alternative embodiment, DNA can be adhered to particles, such as gold particles.

Minigenes can also be delivered using other bacterial or viral delivery systems well known in the art, *e.g.*, an expression construct encoding epitopes of the invention can be incorporated into a viral vector such as vaccinia.

IV.K.2. Combinations of CTL Peptides with Helper Peptides

Vaccine compositions comprising the peptides of the present invention, or analogs thereof, which have immunostimulatory activity may be modified to provide desired attributes, such as improved serum half-life, or to enhance immunogenicity.

For instance, the ability of a peptide to induce CTL activity can be enhanced by linking the peptide to a sequence which contains at least one epitope that is capable of inducing a T helper cell response. The use of T helper epitopes in conjunction with CTL epitopes to enhance immunogenicity is illustrated, for example, in the co-pending applications U.S.S.N. 08/820,360, U.S.S.N. 08/197,484, and U.S.S.N. 08/464,234.

Although a CTL peptide can be directly linked to a T helper peptide, often CTL epitope/HTL epitope conjugates are linked by a spacer molecule. The spacer is typically comprised of relatively small, neutral molecules, such as amino acids or amino acid mimetics, which are substantially uncharged under physiological conditions. The spacers are typically selected from, *e.g.*, Ala, Gly, or other neutral spacers of nonpolar amino acids or neutral polar amino acids. It will be understood that the optionally present spacer need not be comprised of the same residues and thus may be a hetero- or homo-oligomer. When present, the spacer will usually be at least one or two residues, more usually three to six residues and sometimes 10 or more residues. The CTL peptide epitope can be linked to the T helper peptide epitope either directly or via a spacer either at the amino or carboxy terminus of the CTL peptide. The amino terminus of either the immunogenic peptide or the T helper peptide may be acylated.

In certain embodiments, the T helper peptide is one that is recognized by T helper cells present in the majority of the population. This can be accomplished by selecting amino acid sequences that bind to many, most, or all of the HLA class II molecules. These are known as "loosely HLA-restricted" or "promiscuous" T helper sequences. Examples of peptides that are promiscuous include sequences from antigens such as tetanus toxoid at positions 830-843 (QYIKANSKFIGITE), *Plasmodium falciparum* circumsporozoite (CS) protein at positions 378-398 (DIEKKIAKMEKASSVFNVVNS), and *Streptococcus*

18kD protein at positions 116 (GAVDSILGGVATYGAA). Other examples include peptides bearing a DR 1-4-7 supermotif, or either of the DR3 motifs.

Alternatively, it is possible to prepare synthetic peptides capable of stimulating T helper lymphocytes, in a loosely HLA-restricted fashion, using amino acid sequences not found in nature (*see, e.g.*, PCT publication WO 95/07707). These synthetic compounds called Pan-DR-binding epitopes (*e.g.*, PADRE™, Epimmune, Inc., San Diego, CA) are designed to most preferrably bind most HLA-DR (human HLA class II) molecules. For instance, a pan-DR-binding epitope peptide having the formula: aKXVAAWTLKAAa, where "X" is either cyclohexylalanine, phenylalanine, or tyrosine, and "a" is either D-alanine or L-alanine, has been found to bind to most HLA-DR alleles, and to stimulate the response of T helper lymphocytes from most individuals, regardless of their HLA type. An alternative of a pan-DR binding epitope comprises all "L" natural amino acids and can be provided in the form of nucleic acids that encode the epitope.

HTL peptide epitopes can also be modified to alter their biological properties. For example, they can be modified to include D-amino acids to increase their resistance to proteases and thus extend their serum half life, or they can be conjugated to other molecules such as lipids, proteins, carbohydrates, and the like to increase their biological activity. For example, a T helper peptide can be conjugated to one or more palmitic acid chains at either the amino or carboxyl termini.

IV.K.3. Combinations of CTL Peptides with T Cell Priming Agents

In some embodiments it may be desirable to include in the pharmaceutical compositions of the invention at least one component which primes cytotoxic T lymphocytes. Lipids have been identified as agents capable of priming CTL *in vivo* against viral antigens. For example, palmitic acid residues can be attached to the ε-and α- amino groups of a lysine residue and then linked, *e.g.*, via one or more linking residues such as Gly, Gly-Gly-, Ser, Ser-Ser, or the like, to an immunogenic peptide. The lipidated peptide can then be administered either directly in a micelle or particle, incorporated into a liposome, or emulsified in an adjuvant, *e.g.*, incomplete Freund's adjuvant. A preferred immunogenic composition comprises palmitic acid attached to ε- and α- amino groups of Lys, which is attached via linkage, *e.g.*, Ser-Ser, to the amino terminus of the immunogenic peptide.

As another example of lipid priming of CTL responses, *E. coli* lipoproteins, such as tripalmitoyl-S-glycerylcysteinlyseryl- serine (P_3CSS) can be used to prime virus specific CTL when covalently attached to an appropriate peptide (*see, e.g.*, Deres, *et al., Nature* 342:561, 1989). Peptides of the invention can be coupled to P_3CSS, for example, and the lipopeptide administered to an individual to specifically prime a CTL response to the target antigen. Moreover, because the induction of neutralizing antibodies can also be primed with P_3CSS-conjugated epitopes, two such compositions can be combined to more effectively elicit both humoral and cell-mediated responses.

CTL and/or HTL peptides can also be modified by the addition of amino acids to the termini of a peptide to provide for ease of linking peptides one to another, for coupling to a carrier support or larger peptide, for modifying the physical or chemical properties of the peptide or oligopeptide, or the like. Amino acids such as tyrosine, cysteine, lysine, glutamic or aspartic acid, or the like, can be introduced at the C- or N-terminus of the peptide or oligopeptide, particularly class I peptides. However, it is to be noted

that modification at the carboxyl terminus of a CTL epitope may, in some cases, alter binding characteristics of the peptide. In addition, the peptide or oligopeptide sequences can differ from the natural sequence by being modified by terminal-NH_2 acylation, *e.g.*, by alkanoyl (C_1-C_{20}) or thioglycolyl acetylation, terminal-carboxyl amidation, *e.g.*, ammonia, methylamine, *etc.* In some instances these modifications may provide sites for linking to a support or other molecule.

IV.K.4. Vaccine Compositions Comprising DC Pulsed with CTL and/or HTL Peptides

An embodiment of a vaccine composition in accordance with the invention comprises *ex vivo* administration of a cocktail of epitope-bearing peptides to PBMC, or isolated DC therefrom, from the patient's blood. A pharmaceutical to facilitate harvesting of DC can be used, such as Progenipoietin™ (Monsanto, St. Louis, MO) or GM-CSF/IL-4. After pulsing the DC with peptides and prior to reinfusion into patients, the DC are washed to remove unbound peptides. In this embodiment, a vaccine comprises peptide-pulsed DCs which present the pulsed peptide epitopes complexed with HLA molecules on their surfaces.

The DC can be pulsed *ex vivo* with a cocktail of peptides, some of which stimulate CTL response to one or more antigens of interest, *e.g.*, CEA, p53, Her2/neu, MAGE, prostate cancer-associated antigens and the like. Optionally, a helper T cell peptide such as a PADRE™ family molecule, can be included to facilitate the CTL response.

IV.L. Administration of Vaccines for Therapeutic or Prophylactic Purposes

The peptides of the present invention and pharmaceutical and vaccine compositions of the invention are typically used therapeutically to treat cancer. Vaccine compositions containing the peptides of the invention are typically administered to a cancer patient who has a malignancy associated with expression of one or more tumor-associated antigens. Alternatively, vaccine compositions can be administered to an individual susceptible to, or otherwise at risk for developing a particular type of cancer, *e.g.*, breast cancer.

In therapeutic applications, peptide and/or nucleic acid compositions are administered to a patient in an amount sufficient to elicit an effective CTL and/or HTL response to the tumor antigen and to cure or at least partially arrest or slow symptoms and/or complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, *e.g.*, the particular composition administered, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician.

As noted above, peptides comprising CTL and/or HTL epitopes of the invention induce immune responses when presented by HLA molecules and contacted with a CTL or HTL specific for an epitope comprised by the peptide. The manner in which the peptide is contacted with the CTL or HTL is not critical to the invention. For instance, the peptide can be contacted with the CTL or HTL either *in vivo* or *in vitro*. If the contacting occurs *in vivo*, the peptide itself can be administered to the patient, or other vehicles, *e.g.*, DNA vectors encoding one or more peptides, viral vectors encoding the peptide(s), liposomes and the like, can be used. as described herein.

When the peptide is contacted *in vitro*, the vaccinating agent can comprise a population of cells, *e.g.*, peptide-pulsed dendritic cells, or TAA-specific CTLs, which have been induced by pulsing antigen-presenting cells *in vitro* with the peptide. Such a cell population is subsequently administered to a patient in a therapeutically effective dose.

For pharmaceutical compositions, the immunogenic peptides of the invention, or DNA encoding them, are generally administered to an individual already diagnosed with cancer. The peptides or DNA encoding them can be administered individually or as fusions of one or more peptide sequences.

For therapeutic use, administration should generally begin at the first diagnosis of cancer. This is followed by boosting doses until at least symptoms are substantially abated and for a period thereafter. The embodiment of the vaccine composition (i.e., including, but not limited to embodiments such as peptide cocktails, polyepitopic polypeptides, minigenes, or TAA-specific CTLs) delivered to the patient may vary according to the stage of the disease. For example, a vaccine comprising TAA-specific CTLs may be more efficacious in killing tumor cells in patients with advanced disease than alternative embodiments.

The vaccine compositions of the invention may also be used therapeutically in combination with treatments such as surgery. An example is a situation in which a patient has undergone surgery to remove a primary tumor and the vaccine is then used to slow or prevent recurrence and/or metastasis.

Where susceptible individuals, *e.g.*, individuals who may be diagnosed as being genetically pre-disposed to developing a particular type of tumor, for example breast cancer, are identified prior to diagnosis of cancer, the composition can be targeted to them, thus minimizing the need for administration to a larger population.

The dosage for an initial immunization generally occurs in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1,000 μg and the higher value is about 10,000; 20,000; 30,000; or 50,000 μg. Dosage values for a human typically range from about 500 μg to about 50,000 μg per 70 kilogram patient. Boosting dosages of between about 1.0 μg to about 50,000 μg of peptide pursuant to a boosting regimen over weeks to months may be administered depending upon the patient's response and condition as determined by measuring the specific activity of CTL and HTL obtained from the patient's blood.

Administration should continue until at least clinical symptoms or laboratory tests indicate that the tumor has been eliminated or that the tumor cell burden has been substantially reduced and for a period thereafter. The dosages, routes of administration, and dose schedules are adjusted in accordance with methodologies known in the art.

In certain embodiments, peptides and compositions of the present invention are employed in serious disease states, that is, life-threatening or potentially life threatening situations. In such cases, as a result of the minimal amounts of extraneous substances and the relative nontoxic nature of the peptides in preferred compositions of the invention, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these peptide compositions relative to these stated dosage amounts.

The pharmaceutical compositions for therapeutic treatment are intended for parenteral, topical, oral, intrathecal, or local administration. Preferably, the pharmaceutical compositions are administered parentally, *e.g.*, intravenously, subcutaneously, intradermally, or intramuscularly. Thus, the invention provides compositions for parenteral administration which comprise a solution of the immunogenic peptides dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of

aqueous carriers may be used, *e.g.*, water, buffered water, 0.8% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH-adjusting and buffering agents, tonicity adjusting agents, wetting agents, preservatives, and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, *etc.*

The concentration of peptides of the invention in the pharmaceutical formulations can vary widely, *i.e.*, from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, *etc.*, in accordance with the particular mode of administration selected.

A human unit dose form of the peptide composition is typically included in a pharmaceutical composition that comprises a human unit dose of an acceptable carrier, preferably an aqueous carrier, and is administered in a volume of fluid that is known by those of skill in the art to be used for administration of such compositions to humans (*see*, *e.g.*, Remington's Pharmaceutical Sciences, 17th Edition, A. Gennaro, Editor, Mack Publishing Co., Easton, Pennsylvania, 1985).

The peptides of the invention may also be administered via liposomes, which serve to target the peptides to a particular tissue, such as lymphoid tissue, or to target selectively to infected cells, as well as to increase the half-life of the peptide composition. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations, the peptide to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to a receptor prevalent among lymphoid cells, such as monoclonal antibodies which bind to the CD45 antigen, or with other therapeutic or immunogenic compositions. Thus, liposomes either filled or decorated with a desired peptide of the invention can be directed to the site of lymphoid cells, where the liposomes then deliver the peptide compositions. Liposomes for use in accordance with the invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, *e.g.*, liposome size, acid lability and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, *e.g.*, Szoka, *et al.*, *Ann. Rev. Biophys. Bioeng.* 9:467 (1980), and U.S. Patent Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

For targeting cells of the immune system, a ligand to be incorporated into the liposome can include, *e.g.*, antibodies or fragments thereof specific for cell surface determinants of the desired immune system cells. A liposome suspension containing a peptide may be administered intravenously, locally, topically, *etc.* in a dose which varies according to, *inter alia*, the manner of administration, the peptide being delivered, and the stage of the disease being treated.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such

as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more peptides of the invention, and more preferably at a concentration of 25%-75%.

For aerosol administration, the immunogenic peptides are preferably supplied in finely divided form along with a surfactant and propellant. Typical percentages of peptides are 0.01%-20% by weight, preferably 1%-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1%-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included, as desired, as with, *e.g.*, lecithin for intranasal delivery.

IV.M. HLA EXPRESSION: IMPLICATIONS FOR T CELL-BASED IMMUNOTHERAPY

Disease progression in cancer and infectious disease

It is well recognized that a dynamic interaction between exists between host and disease, both in the cancer and infectious disease settings. In the infectious disease setting, it is well established that pathogens evolve during disease. The strains that predominate early in HIV infection are different from the ones that are associated with AIDS and later disease stages (NS versus S strains). It has long been hypothesized that pathogen forms that are effective in establishing infection may differ from the ones most effective in terms of replication and chronicity.

Similarly, it is widely recognized that the pathological process by which an individual succumbs to a neoplastic disease is complex. During the course of disease, many changes occur in cancer cells. The tumor accumulates alterations which are in part related to dysfunctional regulation of growth and differentiation, but also related to maximizing its growth potential, escape from drug treatment and/or the body's immunosurveillance. Neoplastic disease results in the accumulation of several different biochemical alterations of cancer cells, as a function of disease progression. It also results in significant levels of intra- and inter- cancer heterogeneity, particularly in the late, metastatic stage.

Familiar examples of cellular alterations affecting treatment outcomes include the outgrowth of radiation or chemotherapy resistant tumors during the course of therapy. These examples parallel the emergence of drug resistant viral strains as a result of aggressive chemotherapy, *e.g.*, of chronic HBV and HIV infection, and the current resurgence of drug resistant organisms that cause Tuberculosis and Malaria. It appears that significant heterogeneity of responses is also associated with other approaches to cancer therapy, including anti-angiogenesis drugs, passive antibody immunotherapy, and active T cell-based immunotherapy. Thus, in view of such phenomena, epitopes from multiple disease-related antigens can be used in vaccines and therapeutics thereby counteracting the ability of diseased cells to mutate and escape treatment.

The interplay between disease and the immune system

One of the main factors contributing to the dynamic interplay between host and disease is the immune response mounted against the pathogen, infected cell, or malignant cell. In many conditions such

immune responses control the disease. Several animal model systems and prospective studies of natural infection in humans suggest that immune responses against a pathogen can control the pathogen, prevent progression to severe disease and/or eliminate the pathogen. A common theme is the requirement for a multispecific T cell response, and that narrowly focused responses appear to be less effective. These observations guide skilled artisan as to embodiments of methods and compositions of the present invention that provide for a broad immune response.

In the cancer setting there are several findings that indicate that immune responses can impact neoplastic growth:

First, the demonstration in many different animal models, that anti-tumor T cells, restricted by MHC class I, can prevent or treat tumors.

Second, encouraging results have come from immunotherapy trials.

Third, observations made in the course of natural disease correlated the type and composition of T cell infiltrate within tumors with positive clinical outcomes (Coulie PG, *et al.* Antitumor immunity at work in a melanoma patient In *Advances in Cancer Research*, 213-242, 1999).

Finally, tumors commonly have the ability to mutate, thereby changing their immunological recognition. For example, the presence of monospecific CTL was also correlated with control of tumor growth, until antigen loss emerged (Riker A, *et al.*, Immune selection after antigen-specific immunotherapy of melanoma *Surgery*, Aug: 126(2):112-20, 1999; Marchand M, *et al.*, Tumor regressions observed in patients with metastatic melanoma treated with an antigenic peptide encoded by gene MAGE-3 and presented by HLA-A1 *Int. J. Cancer* 80(2):219-30, Jan. 18, 1999). Similarly, loss of beta 2 microglobulin was detected in 5/13 lines established from melanoma patients after receiving immunotherapy at the NCI (Restifo NP, *et al.*, Loss of functional Beta2 - microglobulin in metastatic melanomas from five patients receiving immunotherapy *Journal of the National Cancer Institute*, Vol. 88 (2), 100-108, Jan. 1996). It has long been recognized that HLA class I is frequently altered in various tumor types. This has led to a hypothesis that this phenomenon might reflect immune pressure exerted on the tumor by means of class I restricted CTL. The extent and degree of alteration in HLA class I expression appears to be reflective of past immune pressures, and may also have prognostic value (van Duinen SG, *et al.*, Level of HLA antigens in locoregional metastases and clinical course of the disease in patients with melanoma *Cancer Research* 48, 1019-1025, Feb. 1988; Möller P, *et al.*, Influence of major histocompatibility complex class I and II antigens on survival in colorectal carcinoma *Cancer Research* 51, 729-736, Jan. 1991). Taken together, these observations provide a rationale for immunotherapy of cancer and infectious disease, and suggest that effective strategies need to account for the complex series of pathological changes associated with disease.

The three main types of alterations in HLA expression in tumors and their functional significance

The level and pattern of expression of HLA class I antigens in tumors has been studied in many different tumor types and alterations have been reported in all types of tumors studied. The molecular mechanisms underlining HLA class I alterations have been demonstrated to be quite heterogeneous. They include alterations in the TAP/processing pathways, mutations of β2-microglobulin and specific HLA heavy chains, alterations in the regulatory elements controlling over class I expression and loss of entire chromosome sections. There are several reviews on this topic, *see, e.g.*, : Garrido F, *et al.*, Natural history

of HLA expression during tumour development *Immunol Today* 14(10):491-499, 1993; Kaklamanis L, *et al.*, Loss of HLA class-I alleles, heavy chains and β2-microglobulin in colorectal cancer *Int. J. Cancer*, 51(3):379-85, May 28,1992. There are three main types of HLA Class I alteration (complete loss, allele-specific loss and decreased expression). The functional significance of each alteration is discussed separately:

Complete loss of HLA expression

Complete loss of HLA expression can result from a variety of different molecular mechanisms, reviewed in (Algarra I, *et al.*, The HLA crossroad in tumor immunology *Human Immunology* 61, 65-73, 2000; Browning M, *et al.*, Mechanisms of loss of HLA class I expression on colorectal tumor cells *Tissue Antigens* 47:364-371, 1996; Ferrone S, et al., Loss of HLA class I antigens by melanoma cells: molecular mechanisms, functional significance and clinical relevance *Immunology Today*, 16(10): 487-494, 1995; Garrido F, *et al.*, Natural history of HLA expression during tumour development *Immunology Today* 14(10):491-499, 1993; Tait, BD, HLA Class I expression on human cancer cells: Implications for effective immunotherapy *Hum Immunol* 61, 158-165, 2000). In functional terms, this type of alteration has several important implications.

While the complete absence of class I expression will eliminate CTL recognition of those tumor cells, the loss of HLA class I will also render the tumor cells extraordinary sensitive to lysis from NK cells (Ohnmacht, GA, *et al.*, Heterogeneity in expression of human leukocyte antigens and melanoma-associated antigens in advanced melanoma *J Cellular Phys* 182:332-338, 2000; Liunggren HG, *et al.*, Host resistance directed selectively against H-2 deficient lymphoma variants: Analysis of the mechanism *J. Exp. Med.*, Dec 1;162(6):1745-59, 1985; Maio M, *et al.*, Reduction in susceptibility to natural killer cell-mediated lysis of human FO-1 melanoma cells after induction of HLA class I antigen expression by transfection with B2m gene *J. Clin. Invest.* 88(1):282-9, July 1991; Schrier PI, *et al.*, Relationship between myc oncogene activation and MHC class I expression *Adv. Cancer Res.*, 60:181-246, 1993).

The complementary interplay between loss of HLA expression and gain in NK sensitivity is exemplified by the classic studies of Coulie and coworkers (Coulie, PG, *et al.*, Antitumor immunity at work in a melanoma patient. In *Advances in Cancer Research*, 213-242, 1999) which described the evolution of a patient's immune response over the course of several years. Because of increased sensitivity to NK lysis, it is predicted that approaches leading to stimulation of innate immunity in general and NK activity in particular would be of special significance. An example of such approach is the induction of large amounts of dendritic cells (DC) by various hematopoietic growth factors, such as Flt3 ligand or ProGP. The rationale for this approach resides in the well known fact that dendritic cells produce large amounts of IL-12, one of the most potent stimulators for innate immunity and NK activity in particular. Alternatively, IL-12 is administered directly, or as nucleic acids that encode it. In this light, it is interesting to note that Flt3 ligand treatment results in transient tumor regression of a class I negative prostate murine cancer model (Ciavarra RP, *et al.*, Flt3-Ligand induces transient tumor regression in an ectopic treatment model of major histocompatibility complex-negative prostate cancer *Cancer Res* 60:2081-84, 2000). In this context, specific anti-tumor vaccines in accordance with the invention synergize with these types of hematopoietic growth factors to facilitate both CTL and NK cell responses, thereby appreciably impairing a cell's ability

to mutate and thereby escape efficacious treatment. Thus, an embodiment of the present invention comprises a composition of the invention together with a method or composition that augments functional activity or numbers of NK cells. Such an embodiment can comprise a protocol that provides a composition of the invention sequentially with an NK-inducing modality, or contemporaneous with an NK-inducing modality.

Secondly, complete loss of HLA frequently occurs only in a fraction of the tumor cells, while the remainder of tumor cells continue to exhibit normal expression. In functional terms, the tumor would still be subject, in part, to direct attack from a CTL response; the portion of cells lacking HLA subject to an NK response. Even if only a CTL response were used, destruction of the HLA expressing fraction of the tumor has dramatic effects on survival times and quality of life.

It should also be noted that in the case of heterogeneous HLA expression, both normal HLA-expressing as well as defective cells are predicted to be susceptible to immune destruction based on "bystander effects." Such effects were demonstrated, e.g., in the studies of Rosendahl and colleagues that investigated in vivo mechanisms of action of antibody targeted superantigens (Rosendahl A, *et al.*, Perforin and IFN-gamma are involved in the antitumor effects of antibody-targeted superantigens *J. Immunol.* 160(11):5309-13, June 1, 1998). The bystander effect is understood to be mediated by cytokines elicited from, *e.g.*, CTLs acting on an HLA-bearing target cell, whereby the cytokines are in the environment of other diseased cells that are concomitantly killed.

Allele-specific loss

One of the most common types of alterations in class I molecules is the selective loss of certain alleles in individuals heterozygous for HLA. Allele-specific alterations might reflect the tumor adaptation to immune pressure, exerted by an immunodominant response restricted by a single HLA restriction element. This type of alteration allows the tumor to retain class I expression and thus escape NK cell recognition, yet still be susceptible to a CTL-based vaccine in accordance with the invention which comprises epitopes corresponding to the remaining HLA type. Thus, a practical solution to overcome the potential hurdle of allele-specific loss relies on the induction of multispecific responses. Just as the inclusion of multiple disease-associated antigens in a vaccine of the invention guards against mutations that yield loss of a specific disease antigens, simultaneously targeting multiple HLA specificities and multiple disease-related antigens prevents disease escape by allele-specific losses.

Decrease in expression (allele-specific or not)

The sensitivity of effector CTL has long been demonstrated (Brower, RC, *et al.*, Minimal requirements for peptide mediated activation of CD8+ CTL *Mol. Immunol.*, 31;1285-93, 1994; Chriustnick, ET, *et al.* Low numbers of MHC class I-peptide complexes required to trigger a T cell response *Nature* 352:67-70, 1991; Sykulev, Y, *et al.*, Evidence that a single peptide-MHC complex on a target cell can elicit a cytolytic T cell response *Immunity*, 4(6):565-71, June 1996). Even a single peptide/MHC complex can result in tumor cells lysis and release of anti-tumor lymphokines. The biological significance of decreased HLA expression and possible tumor escape from immune recognition is not fully known. Nevertheless, it

has been demonstrated that CTL recognition of as few as one MHC/peptide complex is sufficient to lead to tumor cell lysis.

Further, it is commonly observed that expression of HLA can be upregulated by gamma IFN, commonly secreted by effector CTL. Additionally, HLA class I expression can be induced in vivo by both alpha and beta IFN (Halloran, *et al.* Local T cell responses induce widespread MHC expression. *J Immunol* 148:3837, 1992; Pestka, S, *et al.*, Interferons and their actions *Annu. Rev. Biochem.* 56:727-77, 1987). Conversely, decreased levels of HLA class I expression also render cells more susceptible to NK lysis.

With regard to gamma IFN, Torres et al (Torres, MJ, *et al.*, Loss of an HLA haplotype in pancreas cancer tissue and its corresponding tumor derived cell line. *Tissue Antigens* 47:372-81, 1996) note that HLA expression is upregulated by gamma IFN in pancreatic cancer, unless a total loss of haplotype has occurred. Similarly, Rees and Mian note that allelic deletion and loss can be restored, at least partially, by cytokines such as IFN-gamma (Rees, R., *et al.* Selective MHC expression in tumours modulates adaptive and innate antitumour responses *Cancer Immunol Immunother* 48:374-81, 1999). It has also been noted that IFN-gamma treatment results in upregulation of class I molecules in the majority of the cases studied (Browning M, *et al.*, Mechanisms of loss of HLA class I expression on colorectal tumor cells. *Tissue Antigens* 47:364-71, 1996). Kaklamakis, et al. also suggested that adjuvant immunotherapy with IFN-gamma may be beneficial in the case of HLA class I negative tumors (Kaklamanis L, Loss of transporter in antigen processing 1 transport protein and major histocompatibility complex class I molecules in metastatic versus primary breast cancer. *Cancer Research* 55:5191-94, November 1995). It is important to underline that IFN-gamma production is induced and self-amplified by local inflammation/immunization (Halloran, *et al.* Local T cell responses induce widespread MHC expression *J. Immunol* 148:3837, 1992), resulting in large increases in MHC expressions even in sites distant from the inflammatory site.

Finally, studies have demonstrated that decreased HLA expression can render tumor cells more susceptible to NK lysis (Ohnmacht, GA, *et al.*, Heterogeneity in expression of human leukocyte antigens and melanoma-associated antigens in advanced melanoma *J Cellular Phys* 182:332-38, 2000; Liunggren HG, *et al.*, Host resistance directed selectively against H-2 deficient lymphoma variants: Analysis of the mechanism *J. Exp. Med.*, 162(6):1745-59, December 1, 1985; Maio M, *et al.*, Reduction in susceptibility to natural killer cell-mediated lysis of human FO-1 melanoma cells after induction of HLA class I antigen expression by transfection with β2m gene *J. Clin. Invest.* 88(1):282-9, July 1991; Schrier PI, *et al.*, Relationship between myc oncogene activation and MHC class I expression *Adv. Cancer Res.*, 60:181-246, 1993). If decreases in HLA expression benefit a tumor because it facilitates CTL escape, but render the tumor susceptible to NK lysis, then a minimal level of HLA expression that allows for resistance to NK activity would be selected for (Garrido F, *et al.*, Implications for immunosurveillance of altered HLA class I phenotypes in human tumours *Immunol Today* 18(2):89-96, February 1997). Therefore, a therapeutic compositions or methods in accordance with the invention together with a treatment to upregulate HLA expression and/or treatment with high affinity T-cells renders the tumor sensitive to CTL destruction.

Frequency of alterations in HLA expression

The frequency of alterations in class I expression is the subject of numerous studies (Algarra I, *et al.*, The HLA crossroad in tumor immunology *Human Immunology* 61, 65-73, 2000). Rees and Mian

estimate allelic loss to occur overall in 3-20% of tumors, and allelic deletion to occur in 15-50% of tumors. It should be noted that each cell carries two separate sets of class I genes, each gene carrying one HLA-A and one HLA-B locus. Thus, fully heterozygous individuals carry two different HLA-A molecules and two different HLA-B molecules. Accordingly, the actual frequency of losses for any specific allele could be as little as one quarter of the overall frequency. They also note that, in general, a gradient of expression exists between normal cells, primary tumors and tumor metastasis. In a study from Natali and coworkers (Natali PG, *et al.*, Selective changes in expression of HLA class I polymorphic determinants in human solid tumors *PNAS USA* 86:6719-6723, September 1989), solid tumors were investigated for total HLA expression, using W6/32 antibody, and for allele-specific expression of the A2 antigen, as evaluated by use of the BB7.2 antibody. Tumor samples were derived from primary cancers or metastasis, for 13 different tumor types, and scored as negative if less than 20%, reduced if in the 30-80% range, and normal above 80%. All tumors, both primary and metastatic, were HLA positive with W6/32. In terms of A2 expression, a reduction was noted in 16.1 % of the cases, and A2 was scored as undetectable in 39.4 % of the cases. Garrido and coworkers (Garrido F, *et al.*, Natural history of HLA expression during tumour development *Immunol Today* 14(10):491-99, 1993) emphasize that HLA changes appear to occur at a particular step in the progression from benign to most aggressive. Jiminez *et al* (Jiminez P, *et al.*, Microsatellite instability analysis in tumors with different mechanisms for total loss of HLA expression. *Cancer Immunol* Immunother 48:684-90, 2000) have analyzed 118 different tumors (68 colorectal, 34 laryngeal and 16 melanomas). The frequencies reported for total loss of HLA expression were 11% for colon, 18% for melanoma and 13 % for larynx. Thus, HLA class I expression is altered in a significant fraction of the tumor types, possibly as a reflection of immune pressure, or simply a reflection of the accumulation of pathological changes and alterations in diseased cells.

Immunotherapy in the context of HLA loss

A majority of the tumors express HLA class I, with a general tendency for the more severe alterations to be found in later stage and less differentiated tumors. This pattern is encouraging in the context of immunotherapy, especially considering that: 1) the relatively low sensitivity of immunohistochemical techniques might underestimate HLA expression in tumors; 2) class I expression can be induced in tumor cells as a result of local inflammation and lymphokine release; and, 3) class I negative cells are sensitive to lysis by NK cells.

Accordingly, various embodiments of the present invention can be selected in view of the fact that there can be a degree of loss of HLA molecules, particularly in the context of neoplastic disease. For example, the treating physician can assay a patient's tumor to ascertain whether HLA is being expressed. If a percentage of tumor cells express no class I HLA, then embodiments of the present invention that comprise methods or compositions that elicit NK cell responses can be employed. As noted herein, such NK-inducing methods or composition can comprise a Flt3 ligand or ProGP which facilitate mobilization of dendritic cells, the rationale being that dendritic cells produce large amounts of IL-12. IL-12 can also be administered directly in either amino acid or nucleic acid form. It should be noted that compositions in accordance with the invention can be administered concurrently with NK cell-inducing compositions, or these compositions can be administered sequentially.

In the context of allele-specific HLA loss, a tumor retains class I expression and may thus escape NK cell recognition, yet still be susceptible to a CTL-based vaccine in accordance with the invention which comprises epitopes corresponding to the remaining HLA type. The concept here is analogous to embodiments of the invention that include multiple disease antigens to guard against mutations that yield loss of a specific antigen. Thus, one can simultaneously target multiple HLA specificities and epitopes from multiple disease-related antigens to prevent tumor escape by allele-specific loss as well as disease-related antigen loss. In addition, embodiments of the present invention can be combined with alternative therapeutic compositions and methods. Such alternative compositions and methods comprise, without limitation, radiation, cytotoxic pharmaceuticals, and/or compositions/methods that induce humoral antibody responses.

Moreover, it has been observed that expression of HLA can be upregulated by gamma IFN, which is commonly secreted by effector CTL, and that HLA class I expression can be induced in vivo by both alpha and beta IFN. Thus, embodiments of the invention can also comprise alpha, beta and/or gamma IFN to facilitate upregualtion of HLA.

IV.N. REPRIEVE PERIODS FROM THERAPIES THAT INDUCE SIDE EFFECTS: "Scheduled Treatment Interruptions or Drug Holidays"

Recent evidence has shown that certain patients infected with a pathogen, whom are initially treated with a therapeutic regimen to reduce pathogen load, have been able to maintain decreased pathogen load when removed from the therapeutic regimen, *i.e.*, during a "drug holiday" (Rosenberg, E., *et al.*, Immune control of HIV-1 after early treatment of acute infection Nature 407:523-26, Sept. 28, 2000) As appreciated by those skilled in the art, many therapeutic regimens for both pathogens and cancer have numerous, often severe, side effects. During the drug holiday, the patient's immune system keeps the disease in check. Methods for using compositions of the invention are used in the context of drug holidays for cancer and pathogenic infection.

For treatment of an infection, where therapies are not particularly immunosuppressive, compositions of the invention are administered concurrently with the standard therapy. During this period, the patient's immune system is directed to induce responses against the epitopes comprised by the present inventive compositions. Upon removal from the treatment having side effects, the patient is primed to respond to the infectious pathogen should the pathogen load begin to increase. Composition of the invention can be provided during the drug holiday as well.

For patients with cancer, many therapies are immunosuppressive. Thus, upon achievement of a remission or identification that the patient is refractory to standard treatment, then upon removal from the immunosuppressive therapy, a composition in accordance with the invention is administered. Accordingly, as the patient's immune system reconstitutes, precious immune resources are simultaneously directed against the cancer. Composition of the invention can also be administered concurrently with an immunosuppressive regimen if desired.

IV.O. Kits

The peptide and nucleic acid compositions of this invention can be provided in kit form together with instructions for vaccine administration. Typically the kit would include desired peptide compositions in a container, preferably in unit dosage form and instructions for administration. An alternative kit would include a minigene construct with desired nucleic acids of the invention in a container, preferably in unit dosage form together with instructions for administration. Lymphokines such as IL-2 or IL-12 may also be included in the kit. Other kit components that may also be desirable include, for example, a sterile syringe, booster dosages, and other desired excipients.

IV.P. Overview

Epitopes in accordance with the present invention were successfully used to induce an immune response. Immune responses with these epitopes have been induced by administering the epitopes in various forms. The epitopes have been administered as peptides, as nucleic acids, and as viral vectors comprising nucleic acids that encode the epitope(s) of the invention. Upon administration of peptide-based epitope forms, immune responses have been induced by direct loading of an epitope onto an empty HLA molecule that is expressed on a cell, and via internalization of the epitope and processing via the HLA class I pathway; in either event, the HLA molecule expressing the epitope was then able to interact with and induce a CTL response. Peptides can be delivered directly or using such agents as liposomes. They can additionally be delivered using ballistic delivery, in which the peptides are typically in a crystalline form. When DNA is used to induce an immune response, it is administered either as naked DNA, generally in a dose range of approximately 1-5mg, or via the ballistic "gene gun" delivery, typically in a dose range of approximately 10-100 μg. The DNA can be delivered in a variety of conformations, *e.g.*, linear, circular *etc.* Various viral vectors have also successfully been used that comprise nucleic acids which encode epitopes in accordance with the invention.

Accordingly compositions in accordance with the invention exist in several forms. Embodiments of each of these composition forms in accordance with the invention have been successfully used to induce an immune response.

One composition in accordance with the invention comprises a plurality of peptides. This plurality or cocktail of peptides is generally admixed with one or more pharmaceutically acceptable excipients. The peptide cocktail can comprise multiple copies of the same peptide or can comprise a mixture of peptides. The peptides can be analogs of naturally occurring epitopes. The peptides can comprise artificial amino acids and/or chemical modifications such as addition of a surface active molecule, *e.g.*, lipidation; acetylation, glycosylation, biotinylation, phosphorylation etc. The peptides can be CTL or HTL epitopes. In a preferred embodiment the peptide cocktail comprises a plurality of different CTL epitopes and at least one HTL epitope. The HTL epitope can be naturally or non-naturally (e.g., PADRE®, Epimmune Inc., San Diego, CA). The number of distinct epitopes in an embodiment of the invention is generally a whole unit integer from one through two hundred (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 105, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126,

127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200).

An additional embodiment of a composition in accordance with the invention comprises a polypeptide multi-epitope construct, *i.e.*, a polyepitopic peptide. Polyepitopic peptides in accordance with the invention are prepared by use of technologies well-known in the art. By use of these known technologies, epitopes in accordance with the invention are connected one to another. The polyepitopic peptides can be linear or non-linear, *e.g.*, multivalent. These polyepitopic constructs can comprise artificial amino acids, spacing or spacer amino acids, flanking amino acids, or chemical modifications between adjacent epitope units. The polyepitopic construct can be a heteropolymer or a homopolymer. The polyepitopic constructs generally comprise epitopes in a quantity of any whole unit integer between 2-150 (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or, 100). The polyepitopic construct can comprise CTL and/or HTL epitopes. One or more of the epitopes in the construct can be modified, *e.g.*, by addition of a surface active material, *e.g.* a lipid, or chemically modified, *e.g.*, acetylation, *etc.* Moreover, bonds in the multiepitopic construct can be other than peptide bonds, *e.g.*, covalent bonds, ester or ether bonds, disulfide bonds, hydrogen bonds, ionic bonds *etc.*

Alternatively, a composition in accordance with the invention comprises construct which comprises a series, sequence, stretch, *etc.*, of amino acids that have homology to (*i.e.*, corresponds to or is contiguous with) to a native sequence. This stretch of amino acids comprises at least one subsequence of amino acids that, if cleaved or isolated from the longer series of amino acids, functions as an HLA class I or HLA class II epitope in accordance with the invention. In this embodiment, the peptide sequence is modified, so as to become a construct as defined herein, by use of any number of techniques known or to be provided in the art. The polyepitopic constructs can contain homology to a native sequence in any whole unit integer increment from 70-100%, e.g., 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or, 100 percent.

A further embodiment of a composition in accordance with the invention is an antigen presenting cell that comprises one or more epitopes in accordance with the invention. The antigen presenting cell can be a "professional" antigen presenting cell, such as a dendritic cell. The antigen presenting cell can comprise the epitope of the invention by any means known or to be determined in the art. Such means include pulsing of dendritic cells with one or more individual epitopes or with one or more peptides that comprise multiple epitopes, by nucleic acid administration such as ballistic nucleic acid delivery or by other techniques in the art for administration of nucleic acids, including vector-based, *e.g.* viral vector, delivery of nucleic acids.

Further embodiments of compositions in accordance with the invention comprise nucleic acids that encode one or more peptides of the invention, or nucleic acids which encode a polyepitopic peptide in accordance with the invention. As appreciated by one of ordinary skill in the art, various nucleic acids

compositions will encode the same peptide due to the redundancy of the genetic code. Each of these nucleic acid compositions falls within the scope of the present invention. This embodiment of the invention comprises DNA or RNA, and in certain embodiments a combination of DNA and RNA. It is to be appreciated that any composition comprising nucleic acids that will encode a peptide in accordance with the invention or any other peptide based composition in accordance with the invention, falls within the scope of this invention.

It is to be appreciated that peptide-based forms of the invention (as well as the nucleic acids that encode them) can comprise analogs of epitopes of the invention generated using priniciples already known, or to be known, in the art. Principles related to analoging are now known in the art, and are disclosed herein; moreover, analoging principles (heteroclitic analoging) are disclosed in co-pending application serial number U.S.S.N. 09/226,775 filed 6 January 1999. Generally the compositions of the invention are isolated or purified.

The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of non-critical parameters that can be changed or modified to yield alternative embodiments in accordance with the invention.

V. EXAMPLES

The following examples illustrate identification, selection, and use of immunogenic Class I and Class II peptide epitopes for inclusion in vaccine compositions.

Example 1. HLA Class I and Class II Binding Assays

The following example of peptide binding to HLA molecules demonstrates quantification of binding affinities of HLA class I and class II peptides. Binding assays can be performed with peptides that are either motif-bearing or not motif-bearing.

HLA class I and class II binding assays using purified HLA molecules were performed in accordance with disclosed protocols (*e.g.*, PCT publications WO 94/20127 and WO 94/03205; Sidney *et al., Current Protocols in Immunology* 18.3.1 (1998); Sidney, *et al., J. Immunol.* 154:247 (1995); Sette, *et al., Mol. Immunol.* 31:813 (1994)). Briefly, purified MHC molecules (5 to 500nM) were incubated with various unlabeled peptide inhibitors and 1-10nM ^{125}I-radiolabeled probe peptides as described. Following incubation, MHC-peptide complexes were separated from free peptide by gel filtration and the fraction of peptide bound was determined. Typically, in preliminary experiments, each MHC preparation was titered in the presence of fixed amounts of radiolabeled peptides to determine the concentration of HLA molecules necessary to bind 10-20% of the total radioactivity. All subsequent inhibition and direct binding assays were performed using these HLA concentrations.

Since under these conditions [label]<[HLA] and $IC_{50} \geq$[HLA], the measured IC_{50} values are reasonable approximations of the true K_D values. Peptide inhibitors are typically tested at concentrations ranging from 120 μg/ml to 1.2 ng/ml, and are tested in two to four completely independent experiments. To allow comparison of the data obtained in different experiments, a relative binding figure is calculated for each peptide by dividing the IC_{50} of a positive control for inhibition by the IC_{50} for each tested peptide

(typically unlabeled versions of the radiolabeled probe peptide). For database purposes, and inter-experiment comparisons, relative binding values are compiled. These values can subsequently be converted back into IC_{50} nM values by dividing the IC_{50} nM of the positive controls for inhibition by the relative binding of the peptide of interest. This method of data compilation has proven to be the most accurate and consistent for comparing peptides that have been tested on different days, or with different lots of purified MHC.

Binding assays as outlined above can be used to analyze supermotif and/or motif-bearing epitopes as, for example, described in Example 2.

Example 2. Identification of HLA Supermotif- and Motif-Bearing CTL Candidate Epitopes

Vaccine compositions of the invention may include multiple epitopes that comprise multiple HLA supermotifs or motifs to achieve broad population coverage. This example illustrates the identification of supermotif- and motif-bearing epitopes for the inclusion in such a vaccine composition. Calculation of population coverage is performed using the strategy described below.

Computer searches and algorthims for identification of supermotif and/or motif-bearing epitopes

The searches performed to identify the motif-bearing peptide sequences in Examples 2 and 5 employed protein sequence data for the tumor-associated antigen HER2/neu.

Computer searches for epitopes bearing HLA Class I or Class II supermotifs or motifs were performed as follows. All translated protein sequences were analyzed using a text string search software program, *e.g.*, MotifSearch 1.4 (D. Brown, San Diego) to identify potential peptide sequences containing appropriate HLA binding motifs; alternative programs are readily produced in accordance with information in the art in view of the motif/supermotif disclosure herein. Furthermore, such calculations can be made mentally. Identified A2-, A3-, and DR-supermotif sequences were scored using polynomial algorithms to predict their capacity to bind to specific HLA-Class I or Class II molecules. These polynomial algorithms take into account both extended and refined motifs (that is, to account for the impact of different amino acids at different positions), and are essentially based on the premise that the overall affinity (or ΔG) of peptide-HLA molecule interactions can be approximated as a linear polynomial function of the type:

$$\text{“}\Delta G\text{”} = a_{1i} \times a_{2i} \times a_{3i} \ldots\ldots \times a_{ni}$$

where a_{ji} is a coefficient which represents the effect of the presence of a given amino acid (j) at a given position (i) along the sequence of a peptide of n amino acids. The crucial assumption of this method is that the effects at each position are essentially independent of each other (i.e., independent binding of individual side-chains). When residue j occurs at position i in the peptide, it is assumed to contribute a constant amount j_i to the free energy of binding of the peptide irrespective of the sequence of the rest of the peptide. This assumption is justified by studies from our laboratories that demonstrated that peptides are bound to MHC and recognized by T cells in essentially an extended conformation (data omitted herein).

The method of derivation of specific algorithm coefficients has been described in Gulukota *et al.*, *J. Mol. Biol.* 267:1258-126, 1997; (*see* also Sidney *et al.*, *Human Immunol.* 45:79-93, 1996; and Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). Briefly, for all i positions, anchor and non-anchor alike, the geometric mean of the average relative binding (ARB) of all peptides carrying j is calculated relative to the

remainder of the group, and used as the estimate of j_i. For Class II peptides, if multiple alignments are possible, only the highest scoring alignment is utilized, following an iterative procedure. To calculate an algorithm score of a given peptide in a test set, the ARB values corresponding to the sequence of the peptide are multiplied. If this product exceeds a chosen threshold, the peptide is predicted to bind. Appropriate thresholds are chosen as a function of the degree of stringency of prediction desired.

Selection of HLA-A2 supertype cross-reactive peptides

The complete protein sequence from HER2/neu was scanned, utilizing motif identification software, to identify 8-, 9-, 10-, and 11-mer sequences containing the HLA-A2-supermotif main anchor specificity.

A total of 623 HLA-A2 supermotif-positive sequences were identified. Of these, 73 scored positive in the A2 algorithm and the peptides corresponding to the sequences were then synthesized. An additional 90 A2 supermotif-bearing nonamers and decamers were also synthesized. These 163 peptides were then tested for their capacity to bind purified HLA-A*0201 molecules *in vitro* (HLA-A*0201 is considered a prototype A2 supertype molecule). Twenty of the peptides bound A*0201 with IC_{50} values ≤500 nM.

The twenty A*0201-binding peptides were subsequently tested for the capacity to bind to additional A2-supertype molecules (A*0202, A*0203, A*0206, and A*6802). As shown in Table XXII, 9 of the 20 peptides were found to be A2-supertype cross-reactive binders, binding at least three of the five A2-supertype alleles tested.

Selection of HLA-A3 supermotif-bearing epitopes

The protein sequences scanned above are also examined for the presence of peptides with the HLA-A3-supermotif primary anchors using methodology similar to that performed to identify HLA-A2 supermotif-bearing epitopes.

Peptides corresponding to the supermotif-bearing sequences are then synthesized and tested for binding to HLA-A*0301 and HLA-A*1101 molecules, the two most prevalent A3-supertype alleles. The peptides that are found to bind one of the two alleles with binding affinities of ≤500 nM are then tested for binding cross-reactivity to the other common A3-supertype alleles (A*3101, A*3301, and A*6801) to identify those that can bind at least three of the five HLA-A3-supertype molecules tested. Examples of HLA-A3 cross-binding supermotif-bearing peptides identified in accordance with this procedure are provided in Table XXIII.

Selection of HLA-B7 supermotif bearing epitopes

The same target antigen protein sequences are also analyzed to identify HLA-B7-supermotif-bearing sequences. The corresponding peptides are then synthesized and tested for binding to HLA-B*0702, the most common B7-supertype allele (*i.e.*, the prototype B7 supertype allele). Those peptides that bind B*0702 with IC_{50} of ≤500 nM are then tested for binding to other common B7-supertype molecules (B*3501, B*5101, B*5301, and B*5401) to identify those peptides that are capable of binding to three or

more of the five B7-supertype alleles tested. Examples of HLA-B7 cross-binding supermotif-bearing peptides identified in accordance with this procedure are provided in Table XXIV.

Selection of A1 and A24 motif-bearing epitopes

To further increase population coverage, HLA-A1 and -A24 motif-bearing epitopes can also be incorporated into potential vaccine constructs. An analysis of the protein sequence data from the target antigen utilized above is also performed to identify HLA-A1- and A24-motif-containing conserved sequences. The corresponding peptide sequence are then synthesized and tested for binding to the appropriate allele-specific HLA molecule, HLA-A1 or HLA-24. Peptides are identified that bind to the allele-specific HLA molecules at an IC_{50} of ≤500 nM. Examples of peptides identified in accordance with this procedure are provided in Tables XXV and XXVI.

Example 3. Confirmation of Immunogenicity

The nine cross-reactive candidate CTL A2-supermotif-bearing peptides identified in Example 2 were selected for *in vitro* immunogenicity testing. Testing was performed using the following methodology:

Target Cell Lines for Cellular Screening:

The .221A2.1 cell line, produced by transferring the HLA-A2.1 gene into the HLA-A, -B, -C null mutant human B-lymphoblastoid cell line 721.221, was used as the peptide-loaded target to measure activity of HLA-A2.1-restricted CTL. The colon adenocarcinoma cell lines SW403 and HT-29 were obtained from the American Type Culture Collection (ATCC) (Rockville, MD). The cell lines that were obtained from ATCC were maintained under the culture conditions recommended by the supplier. All other cell lines were grown in RPMI-1640 medium supplemented with antibiotics, sodium pyruvate, nonessential amino acids and 10% (v/v) heat inactivated FCS. The colon cancer cells were treated with 100U/ml IFNγ (Genzyme) for 48 hours at 37°C before use as targets in the ^{51}Cr release and *in situ* IFNγ assays.

Primary CTL Induction Cultures:

Generation of Dendritic Cells (DC): PBMCs were thawed in RPMI with 30 μg/ml DNAse, washed twice and resuspended in complete medium (RPMI-1640 plus 5% AB human serum, non-essential amino acids, sodium pyruvate, L-glutamine and penicillin/strpetomycin). The monocytes were purified by plating 10 x 10^6 PBMC/well in a 6-well plate. After 2 hours at 37°C, the non-adherent cells were removed by gently shaking the plates and aspirating the supernatants. The wells were washed a total of three times with 3 ml RPMI to remove most of the non-adherent and loosely adherent cells. Three ml of complete medium containing 50 ng/ml of GM-CSF and 1,000 U/ml of IL-4 were then added to each well. DC were used for CTL induction cultures following 7 days of culture.

Induction of CTL with DC and Peptide: CD8+ T-cells were isolated by positive selection with Dynal immunomagnetic beads (Dynabeads® M-450) and the detacha-bead® reagent. Typically about 200-250x10^6 PBMC were processed to obtain 24x10^6 $CD8^+$ T-cells (enough for a 48-well plate culture). Briefly, the PBMCs were thawed in RPMI with 30μg/ml DNAse, washed once with PBS containing 1%

human AB serum and resuspended in PBS/1% AB serum at a concentration of $20x10^6$cells/ml. The magnetic beads were washed 3 times with PBS/AB serum, added to the cells (140µl beads/$20x10^6$ cells) and incubated for 1 hour at 4°C with continuous mixing. The beads and cells were washed 4x with PBS/AB serum to remove the nonadherent cells and resuspended at $100x10^6$ cells/ml (based on the original cell number) in PBS/AB serum containing 100µl/ml detacha-bead® reagent and 30µg/ml DNAse. The mixture is incubated for 1 hour at room temperature with continuous mixing. The beads were washed again with PBS/AB/DNAse to collect the CD8+ T-cells. The DC were collected and centrifuged at 1300 rpm for 5-7 minutes, washed once with PBS with 1% BSA, counted and pulsed with 40µg/ml of peptide at a cell concentration of $1-2x10^6$/ml in the presence of 3µg/ml $ß_2$- microglobulin for 4 hours at 20°C. The DC were then irradiated (4,200 rads), washed 1 time with medium and counted again.

Setting up induction cultures: 0.25 ml cytokine-generated DC (@$1x10^5$ cells/ml) were co-cultured with 0.25ml of CD8+ T-cells (@$2x10^6$ cell/ml) in each well of a 48-well plate in the presence of 10 ng/ml of IL-7. rHuman IL10 was added the next day at a final concentration of 10 ng/ml and rhuman IL2 was added 48 hours later at 10IU/ml.

Restimulation of the induction cultures with peptide-pulsed adherent cells: Seven and fourteen days after the primary induction the cells were restimulated with peptide-pulsed adherent cells. The PBMCS were thawed and washed twice with RPMI and DNAse. The cells were resuspended at $5x10^6$ cells/ml and irradiated at ~4200 rads. The PBMCs were plated at $2x10^6$ in 0.5ml complete medium per well and incubated for 2 hours at 37°C. The plates were washed twice with RPMI by tapping the plate gently to remove the nonadherent cells and the adherent cells pulsed with 10µg/ml of peptide in the presence of 3 µg/ml $ß_2$ microglobulin in 0.25ml RPMI/5%AB per well for 2 hours at 37°C. Peptide solution from each well was aspirated and the wells were washed once with RPMI. Most of the media was aspirated from the induction cultures (CD8+ cells) and brought to 0.5 ml with fresh media. The cells were then transferred to the wells containing the peptide-pulsed adherent cells. Twenty four hours later rhuman IL10 was added at a final concentration of 10ng/ml and rhuman IL2 was added the next day and again 2-3 days later at 50IU/ml (Tsai *et al.*, *Critical Reviews in Immunology* 18(1-2):65-75, 1998). Seven days later the cultures were assayed for CTL activity in a ^{51}Cr release assay. In some experiments the cultures were assayed for peptide-specific recognition in the in situ IFNγ ELISA at the time of the second restimulation followed by assay of endogenous recognition 7 days later. After expansion, activity was measured in both assays for a side by side comparison.

Measurement of CTL lytic activity by ^{51}Cr release.

Seven days after the second restimulation, cytotoxicity was determined in a standard (5hr) ^{51}Cr release assay by assaying individual wells at a single E:T. Peptide-pulsed targets were prepared by incubating the cells with 10µg/ml peptide overnight at 37°C.

Adherent target cells were removed from culture flasks with trypsin-EDTA. Target cells were labelled with 200µCi of ^{51}Cr sodium chromate (Dupont, Wilmington, DE) for 1 hour at 37°C. Labelled target cells are resuspended at 10^6 per ml and diluted 1:10 with K562 cells at a concentration of $3.3x10^6$/ml (an NK-sensitive erythroblastoma cell line used to reduce non-specific lysis). Target cells (100 µl) and 100µl of effectors were plated in 96 well round-bottom plates and incubated for 5 hours at 37°C. At that time, 100 µl of supernatant were collected from each well and percent lysis was determined according

formula: [(cpm of the test sample- cpm of the spontaneous ^{51}Cr release sample)/(cpm of the maximal ^{51}Cr release sample- cpm of the spontaneous ^{51}Cr release sample)] x 100. Maximum and spontaneous release were determined by incubating the labelled targets with 1% Trition X-100 and media alone, respectively. A positive culture was defined as one in which the specific lysis (sample- background) was 10% or higher in the case of individual wells and was 15% or more at the 2 highest E:T ratios when expanded cultures were assayed.

***In situ* Measurement of Human γIFN Production as an Indicator of Peptide-specific and Endogenous Recognition**

Immulon 2 plates were coated with mouse anti-human IFNγ monoclonal antibody (4 μg/ml 0.1M $NaHCO_3$, pH8.2) overnight at 4°C. The plates were washed with Ca^{2+}, Mg^{2+}-free PBS/0.05% Tween 20 and blocked with PBS/10% FCS for 2 hours, after which the CTLs (100 μl/well) and targets (100 μl/well) were added to each well, leaving empty wells for the standards and blanks (which received media only). The target cells, either peptide-pulsed or endogenous targets, were used at a concentration of $1x10^6$ cells/ml. The plates were incubated for 48 hours at 37°C with 5% CO_2.

Recombinant human IFNγ was added to the standard wells starting at 400 pg or 1200pg/100μl/well and the plate incubated for 2 hours at 37°C. The plates were washed and 100 μl of biotinylated mouse anti-human IFNγ monoclonal antibody (4μg/ml in PBS/3%FCS/0.05% Tween 20) were added and incubated for 2 hours at room temperature. After washing again, 100 μl HRP-streptavidin were added and the plates incubated for 1 hour at room temperature. The plates were then washed 6x with wash buffer, 100μl/well developing solution (TMB 1:1) were added, and the plates allowed to develop for 5-15 minutes. The reaction was stopped with 50 μl/well 1M H_3PO_4 and read at OD450. A culture was considered positive if it measured at least 50 pg of IFNγ/well above background and was twice the background level of expression.

CTL Expansion. Those cultures that demonstrated specific lytic activity against peptide-pulsed targets and/or tumor targets were expanded over a two week period with anti-CD3. Briefly, $5x10^4$ CD8+ cells were added to a T25 flask containing the following: $1x10^6$ irradiated (4,200 rad) PBMC (autologous or allogeneic) per ml, $2x10^5$ irradiated (8,000 rad) EBV- transformed cells per ml, and OKT3 (anti-CD3) at 30ng per ml in RPMI-1640 containing 10% (v/v) human AB serum, non-essential amino acids, sodium pyruvate, 25μM 2-mercaptoethanol, L-glutamine and penicillin/streptomycin. rHuman IL2 was added 24 hours later at a final concentration of 200IU/ml and every 3 days thereafter with fresh media at 50IU/ml. The cells were split if the cell concentration exceeded $1x10^6$/ml and the cultures were assayed between days 13 and 15 at E:T ratios of 30, 10, 3 and 1:1 in the ^{51}Cr release assay or at $1x10^6$/ml in the *in situ* IFNγ assay using the same targets as before the expansion.

Immunogenicity of A2 supermotif-bearing peptides

A2-supermotif cross-reactive binding peptides were tested in the cellular assay for the ability to induce peptide-specific CTL in normal individuals. In this analysis, a peptide was considered to be an epitope if it induced peptide-specific CTLs in at least 2 donors (unless otherwise noted) and if those CTLs also recognized the endogenously expressed peptide. Peptides that were able to induce a peptide-specific CTL response in at least 2 normal donors are shown in Table XXVII. Further analysis demonstrated those

that also recognized target cells pulsed with the wild-type peptide and tumor targets that endogenously express HER2/neu (Table XXVII). An additional wild-type peptide, Her2/neu.5 was selected for evaluation based on its A2.1 binding affinity and, although it binds to only 2 HLA-A2 supertype molecules, it was capable of generating a strong CTL response that was both peptide- and tumor-specific.

Immunogenicity was additionally confirmed using PBMCs isolated from cancer patients. Briefly, PBMCs were isolated from two patients with ovarian cancer, re-stimulated with peptide-pulsed monocytes and assayed for the ability to recognize peptide-pulsed target cells as well as transfected cells endogenously expressing the antigen. These data indicated that Her2/neu.435 was recognized in 2 donors as well as Her2/neu.369, Her2/neu.952, and Her2/neu.48. Her2/neu.689 is also an epitope, but not a supertype binder. Of the other peptides tested, Her2/neu.665 and Her2/neu.773 were recognized by CTLs from only one of the two patients and CTLs to Her2/neu.153 and Her2/neu.789 recognized peptide-pulsed targets only.

*Evaluation of A*03/A11 immunogenicity*

HLA-A3 supermotif-bearing cross-reactive binding peptides are also evaluated for immunogenicity using methodology analogous for that used to evaluate the immunogenicity of the HLA-A2 supermotif peptides. Using this procedure, peptides that induce an immune response are identified. Examples of such peptides are shown in Table XXIII.

Evaluation of immunogenicity of Motif/Supermotif-Bearing Peptides.

Analogous methodology, as appreciated by one of ordinary skill in the art, is employed to determine immunogenicity of peptides bearing HLA class I motifs and/or supermotifs set out herein. Using such a prodcedure peptides that induce an immune response are identified.

Example 4. Implementation of the Extended Supermotif to Improve the Binding Capacity of Native Epitopes by Creating Analogs

HLA motifs and supermotifs (comprising primary and/or secondary residues) are useful in the identification and preparation of highly cross-reactive native peptides, as demonstrated herein. Moreover, the definition of HLA motifs and supermotifs also allows one to engineer highly cross-reactive epitopes by identifying residues within a native peptide sequence which can be analogued, or "fixed" to confer upon the peptide certain characteristics, *e.g.* greater cross-reactivity within the group of HLA molecules that comprise a supertype, and/or greater binding affinity for some or all of those HLA molecules. Examples of analog peptides that exhibit modulated binding affinity are set forth in this example and provided in Tables XXII through XXVII.

Analoguing at Primary Anchor Residues

Peptide engineering strategies were implemented to further increase the cross-reactivity of the epitopes identified above. On the basis of the data disclosed, *e.g.*, in related and co-pending U.S.S.N 09/226,775, the main anchors of A2-supermotif-bearing peptides are altered, for example, to introduce a preferred L, I, V, or M at position 2, and I or V at the C-terminus.

Peptides that exhibit at least weak A*0201 binding (IC_{50} of 5000 nM or less), and carrying suboptimal anchor residues at either position 2, the C-terminal position, or both, can be fixed by introducing canonical substitutions (L at position 2 and V at the C-terminus). Those analogued peptides that show at least a three-fold increase in A*0201 binding and bind with an IC_{50} of 500 nM, or less were then tested for A2 cross-reactive binding along with their wild-type (WT) counterparts. Analogued peptides that bind at least three of the five A2 supertype alleles were then selected for cellular screening analysis (Table XXVII).

Additionally, the selection of analogs for cellular screening analysis was further restricted by the capacity of the WT parent peptide to bind at least weakly, *i.e.*, bind at an IC_{50} of 5000nM or less, to three of more A2 supertype alleles. The rationale for this requirement is that the WT peptides must be present endogenously in sufficient quantity to be biologically relevant. Analogued peptides have been shown to have increased immunogenicity and cross-reactivity by T cells specific for the WT epitope (*see, e.g.*, Parkhurst *et al.*, *J. Immunol.* 157:2539, 1996; and Pogue *et al.*, *Proc. Natl. Acad. Sci. USA* 92:8166, 1995).

In the cellular screening of these peptide analogs, it is important to demonstrate that analog-specific CTLs are also able to recognize the wild-type peptide and, when possible, tumor targets that endogenously express the epitope.

Of the 20 peptides identified in Example 2 that bound to HLA-A*0201 at a high affinity, 15 carried suboptimal primary anchor residues and met the criterion for analoguing at primary anchor residues by introducing a canonical substitution. Analogs of six of the A*0201-binding peptides were created and tested for primary binding to HLA-A*0201 and supertype binding (Table XXII). In 4 of 6 cases, binding to HLA-A*0201 was improved at least three-fold. In 4 cases, crossbinding capability was also improved. In one instance, peptide Her2/neu.153 did not show a three-fold increase in binding to HLA-A*0201, but crossbinding was improved.

Additionally, 22 peptides that weakly bound to HLA-A*0201 that carry suboptimal anchors were also identified and can also be analogued.

Two analogs of Her2/neu.5, two analogs of Her2/neu.369, one version of Her2/neu.952, and one version of Her2/neu.665 were selected for cellular screening studies. As shown in Table XXVII, both Her2/neu.369L2V9 and V2V9 induced peptide-specific CTLs and those CTLs also recognized the target tumor cells expressing that endogenously express the antigen. Her2neu.5B3V9 and Her2/neu.952L2B7V10 induced peptide-specific CTLs in at least 2 donors, but when the positive cultures were expanded, no wild-type peptide or endogenous recognition was observed.

The Her2/neu.665L2V9 analog exhibited binding to four of the five A2 supertype alleles tested, whereas the wildtype peptide only binds two of the five alleles. In the cellular screening analysis, a strong peptide-specific CTL response was observed. The positive cultures were expanded and assayed for peptide and endogenous recognition. Peptide-specific CTL activity was maintained in some of the cultures, but no corresponding endogenous recognition was observed.

Using methodology similar to that used to develop HLA-A2 analogs, analogs of HLA-A3 and HLA-B7 supermotif-bearing epitopes are also generated. For example, peptides binding at least weakly to 3/5 of the A3-supertype molecules can be engineered at primary anchor residues to possess a preferred residue (V, S, M, or A) at position 2. The analog peptides are then tested for the ability to bind A*03 and A*11 (prototype A3 supertype alleles). Those peptides that demonstrate $\leq$ 500 nM binding capacity are

then tested for A3-supertype cross-reactivity. Examples of HLA-A3 supermotif analog peptides are provided in Table XXIII.

B7 supermotif-bearing peptides can, for example, be engineered to possess a preferred residue (V, I, L, or F) at the C-terminal primary anchor position (*see, e.g.* Sidney *et al.* (*J. Immunol.* 157:3480-3490, 1996). Analoged peptides are then tested for cross-reactive binding to B7 supertype alleles. Examples of B7-supermotif-bearing analog peptides are provided in Table XXIV.

Similarly, HLA-A1 and HLA-A24 motif-bearing peptides can be engineered at primary anchor residues to improvde binding to the allele-specific HLA molecule or to improve cross-reactive binding. Examples of analoged HLA-A1 and HLA-A24 motif-bearing peptides are provided in Tables XXV and XXVI.

Analoged peptides that exhibit improved binding and/or or cross-reactivity are evaluated for immunogenicity using methodology similar to that described for the analysis of HLA-A2 supermotif-bearing peptides. Using such a procedure, peptides that induce an immune response are identified, *e.g.* Table XXIII.

Analoguing at Secondary Anchor Residues

Moreover, HLA supermotifs are of value in engineering highly cross-reactive peptides and/or peptides that bind HLA molecules with increased affinity by identifying particular residues at secondary anchor positions that are associated with such properties. Examples of such analoged peptides are provided in Tables XXII-XXIV.

For example, the binding capacity of a B7 supermotif-bearing peptide representing a discreet single amino acid substitution at position 1 can be analyzed. A peptide can, for example, be analogued to substitute L with F at position 1 and subsequently be evaluated for increased binding affinity/ and or increased cross-reactivity. This procedure will identify analogued peptides with modulated binding affinity.

Analoged peptides that exhibit improved binding and/or or cross-reactivity are evaluated for immunogenicity using methodology similar to that described for the analysis of HLA-A2 supermotif-bearing peptides. Using such a procedure, peptides that induce an immune response are identified.

Other analoguing strategies

Another form of peptide analoguing, unrelated to the anchor positions, involves the substitution of a cysteine with α-amino butyric acid. Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally so as to reduce binding capacity. Subtitution of α-amino butyric acid for cysteine not only alleviates this problem, but has been shown to improve binding and crossbinding capabilities in some instances (*see, e.g.*, the review by Sette *et al.*, In: Persistent Viral Infections, Eds. R. Ahmed and I. Chen, John Wiley & Sons, England, 1999).

Analoged peptides that exhibit improved binding and/or or cross-reactivity are evaluated for immunogenicity using methodology similar to that described for the analysis of HLA-A2 supermotif-bearing peptides. Using such a procedure, peptides that induce an immune response are identified.

This Example therefore demonstrates that by the use of even single amino acid substitutions, the binding affinity and/or cross-reactivity of peptide ligands for HLA supertype molecules is modulated.

Example 5. Identification of peptide epitope sequences with HLA-DR binding motifs

Peptide epitopes bearing an HLA class II supermotif or motif may also be identified as outlined below using methodology similar to that described in Examples 1-3.

Selection of HLA-DR-supermotif-bearing epitopes

To identify HLA class II HTL epitopes, the HER2/neu protein sequence was analyzed for the presence of sequences bearing an HLA-DR-motif or supermotif. Specifically, 15-mer sequences were selected comprising a DR-supermotif, further comprising a 9-mer core, and three-residue N- and C-terminal flanking regions (15 amino acids total).

Protocols for predicting peptide binding to DR molecules have been developed (Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). These protocols, specific for individual DR molecules, allow the scoring, and ranking, of 9-mer core regions. Each protocol not only scores peptide sequences for the presence of DR-supermotif primary anchors (i.e., at position 1 and position 6) within a 9-mer core, but additionally evaluates sequences for the presence of secondary anchors. Using allele specific selection tables (see, *e.g.*, Southwood *et al.*, *ibid.*), it has been found that these protocols efficiently select peptide sequences with a high probability of binding a particular DR molecule. Additionally, it has been found that performing these protocols in tandem, specifically those for DR1, DR4w4, and DR7, can efficiently select DR cross-reactive peptides.

The HER2/neu-derived peptides identified above were tested for their binding capacity for various common HLA-DR molecules. All peptides were initially tested for binding to the DR molecules in the primary panel: DR1, DR4w4, and DR7. Peptides binding at least 2 of these 3 DR molecules with an IC_{50} value of 1000 nM or less, were then tested for binding to DR5*0101, DRB1*1501, DRB1*1101, DRB1*0802, and DRB1*1302. Peptides were considered to be cross-reactive DR supertype binders if they bound at an IC_{50} value of 1000 nM or less to at least 5 of the 8 alleles tested.

Following the strategy outlined above, 188 DR supermotif-bearing sequences were identified within the HER2/neu protein sequence. Of those, 41 scored positive in 2 of the 3 combined DR 147 algorithms. These peptides were synthesized and tested for binding to HLA-DRB1*0101, DRB1*0401, DRB1*0701. Of the 41 peptides tested, 18 bound at least 2 of the 3 alleles (Table XXVIII).

These 18 peptides were then tested for binding to secondary DR supertype alleles: DRB5*0101, DRB1*1501, DRB1*1101, DRB1*0802, and DRB1*1302. Nine peptides were identified that bound at least 5 of the 8 alleles tested, of which 8 occurred in distinct, non-overlapping regions (Table XXIX).

Selection of DR3 motif peptides

Because HLA-DR3 is an allele that is prevalent in Caucasian, Black, and Hispanic populations, DR3 binding capacity is an important criterion in the selection of HTL epitopes. However, data generated previously indicated that DR3 only rarely cross-reacts with other DR alleles (Sidney *et al.*, *J. Immunol.* 149:2634-2640, 1992; Geluk *et al.*, *J. Immunol.* 152:5742-5748, 1994; Southwood *et al.*, *J. Immunol.*

160:3363-3373, 1998). This is not entirely surprising in that the DR3 peptide-binding motif appears to be distinct from the specificity of most other DR alleles. For maximum efficiency in developing vaccine candidates it would be desirable for DR3 motifs to be clustered in proximity with DR supermotif regions. Thus, peptides shown to be candidates may also be assayed for their DR3 binding capacity. However, in view of the distinct binding specificity of the DR3 motif, peptides binding only to DR3 can also be considered as candidates for inclusion in a vaccine formulation.

To efficiently identify peptides that bind DR3, the HER2/neu protein sequence was analyzed for conserved sequences carrying one of the two DR3 specific binding motifs (Table III) reported by Geluk *et al.* (*J. Immunol.* 152:5742-5748, 1994). Forty-six motif-positive peptides were identified. The corresponding peptides were then synthesized and tested for the ability to bind DR3 with an affinity of 1000 nM or better, *i.e.*, less than 1000 nM. Seven peptides were found that met this binding criterion (Table XXX), and thereby qualify as HLA class II high affinity binders.

Additionally, the 7 DR3 binders were tested for binding to the DR supertype alleles (Table XXXI). Four of the seven DR3 binders bound at least 3 other DR alleles, and one peptide, Her2/neu.886, was a cross-reactive supertype binder as well. Conversely, the DR supertype cross-reactive binding peptides were also tested for DR3 binding capacity. The cross-reactive DR supermotif-bearing peptides showed little capacity to bind DR3 molecules (Table XXXI).

DR3 binding epitopes identified in this manner may then be included in vaccine compositions with DR supermotif-bearing peptide epitopes.

In summary, 8 DR supertype cross-reactive binding peptides and 7 DR3 binding peptides were identified from the HER2/neu protein sequence, with one peptide shared between the two motifs. Of these, 5 DR supertype and 5 DR3-binding peptides were located in the intracellular domain.

Similarly to the case of HLA class I motif-bearing peptides, the class II motif-bearing peptides may be analogued to improve affinity or cross-reactivity. For example, aspartic acid at position 4 of the 9-mer core sequence is an optimal residue for DR3 binding, and substitution for that residue may improve DR 3 binding. Analoged peptides are evaluated for immunogenicity in accordance with the methodology of Example 6.

Example 6. Immunogenicity of HTL epitopes

This example determines immunogenic DR supermotif- and DR3 motif-bearing epitopes among those identified using the methodology in Example 5. Immunogenicity of HTL epitopes are evaluated in a manner analogous to the determination of immunogenicity of CTL epitopes by assessing the ability to stimulate HTL responses and/or by using appropriate transgenic mouse models. Immunogenicity is determined by screening for: 1.) *in vitro* primary induction using normal PBMC or 2.) recall responses from cancer patient PBMCs.

Example 7. Calculation of phenotypic frequencies of HLA-supertypes in various ethnic backgrounds to determine breadth of population coverage

This example illustrates the assessment of the breadth of population coverage of a vaccine composition comprised of multiple epitopes comprising multiple supermotifs and/or motifs.

In order to analyze population coverage, gene frequencies of HLA alleles were determined. Gene frequencies for each HLA allele were calculated from antigen or allele frequencies utilizing the binomial distribution formulae gf=1-(SQRT(1-af)) (see, *e.g.*, Sidney *et al.*, *Human Immunol.* 45:79-93, 1996). To obtain overall phenotypic frequencies, cumulative gene frequencies were calculated, and the cumulative antigen frequencies derived by the use of the inverse formula [af=1-(1-Cgf)^2].

Where frequency data was not available at the level of DNA typing, correspondence to the serologically defined antigen frequencies was assumed. To obtain total potential supertype population coverage no linkage disequilibrium was assumed, and only alleles confirmed to belong to each of the supertypes were included (minimal estimates). Estimates of total potential coverage achieved by inter-loci combinations were made by adding to the A coverage the proportion of the non-A covered population that could be expected to be covered by the B alleles considered (*e.g.*, total=A+B*(1-A)). Confirmed members of the A3-like supertype are A3, A11, A31, A*3301, and A*6801. Although the A3-like supertype may also include A34, A66, and A*7401, these alleles were not included in overall frequency calculations. Likewise, confirmed members of the A2-like supertype family are A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*6802, and A*6901. Finally, the B7-like supertype-confirmed alleles are: B7, B*3501-03, B51, B*5301, B*5401, B*5501-2, B*5601, B*6701, and B*7801 (potentially also B*1401, B*3504-06, B*4201, and B*5602).

Population coverage achieved by combining the A2-, A3- and B7-supertypes is approximately 86% in five major ethnic groups (see Table XXI). Coverage may be extended by including peptides bearing the A1 and A24 motifs. On average, A1 is present in 12% and A24 in 29% of the population across five different major ethnic groups (Caucasian, North American Black, Chinese, Japanese, and Hispanic). Together, these alleles are represented with an average frequency of 39% in these same ethnic populations. The total coverage across the major ethnicities when A1 and A24 are combined with the coverage of the A2-, A3- and B7-supertype alleles is >95%. An analogous approach can be used to estimate population coverage achieved with combinations of class II motif-bearing epitopes.

Example 8. Recognition Of Generation Of Endogenous Processed Antigens After Priming

This example determines that CTL induced by native or analogued peptide epitopes identified and selected as described in Examples 1-6 recognize endogenously synthesized, *i.e.*, native antigens, using a transgenic mouse model.

Effector cells isolated from transgenic mice that are immunized with peptide epitopes (as described, e.g., in Wentworth et al., *Mol. Immunol.* 32:603, 1995), for example HLA-A2 supermotif-bearing epitopes, are re-stimulated *in vitro* using peptide-coated stimulator cells. Six days later, effector cells are assayed for cytotoxicity and the cell lines that contain peptide-specific cytotoxic activity are further re-stimulated. An additional six days later, these cell lines are tested for cytotoxic activity on ^{51}Cr labeled Jurkat-A2.1/K^b target cells in the absence or presence of peptide, and also tested on ^{51}Cr labeled target cells bearing the endogenously synthesized antigen, *i.e.* cells that are stably transfected with TAA expression vectors.

The result will demonstrate that CTL lines obtained from animals primed with peptide epitope recognize endogenously synthesized antigen. The choice of transgenic mouse model to be used for such an

analysis depends upon the epitope(s) that is being evaluated. In addition to HLA-A*0201/K^b transgenic mice, several other transgenic mouse models including mice with human A11, which may also be used to evaluate A3 epitopes, and B7 alleles have been characterized and others (*e.g.*, transgenic mice for HLA-A1 and A24) are being developed. HLA-DR1 and HLA-DR3 mouse models have also been developed, which may be used to evaluate HTL epitopes.

Example 9. Activity Of CTL-HTL Conjugated Epitopes In Transgenic Mice

This example illustrates the induction of CTLs and HTLs in transgenic mice by use of a tumor associated antigen CTL/HTL peptide conjugate whereby the vaccine composition comprises peptides to be administered to a cancer patient. The peptide composition can comprise multiple CTL and/or HTL epitopes and further, can comprise epitopes selected from multiple-tumor associated antigens. The epitopes are identified using methodology as described in Examples 1-6 This analysis demonstrates the enhanced immunogenicity that can be achieved by inclusion of one or more HTL epitopes in a vaccine composition. Such a peptide composition can comprise an HTL epitope conjugated to a preferred CTL epitope containing, for example, at least one CTL epitope selected from Tables XXVII and XXIII-XXVI, or other analogs of that epitope. The HTL epitope is, for example, selected from Table XXXI. The peptides may be lipidated, if desired.

Immunization procedures: Immunization of transgenic mice is performed as described (Alexander *et al., J. Immunol.* 159:4753-4761, 1997). For example, A2/K^b mice, which are transgenic for the human HLA A2.1 allele and are useful for the assessment of the immunogenicity of HLA-A*0201 motif- or HLA-A2 supermotif-bearing epitopes, are primed subcutaneously (base of the tail) with 0.1 ml of peptide conjugate formulated in saline, or DMSO/saline. Seven days after priming, splenocytes obtained from these animals are restimulated with syngenic irradiated LPS-activated lymphoblasts coated with peptide.

The target cells for peptide-specific cytotoxicity assays are Jurkat cells transfected with the HLA-A2.1/K^b chimeric gene (*e.g.*, Vitiello *et al., J. Exp. Med.* 173:1007, 1991).

In vitro CTL activation: One week after priming, spleen cells ($30x10^6$ cells/flask) are co-cultured at 37°C with syngeneic, irradiated (3000 rads), peptide coated lymphoblasts ($10x10^6$ cells/flask) in 10 ml of culture medium/T25 flask. After six days, effector cells are harvested and assayed for cytotoxic activity.

Assay for cytotoxic activity: Target cells (1.0 to $1.5x10^6$) are incubated at 37°C in the presence of 200 µl of ^{51}Cr. After 60 minutes, cells are washed three times and resuspended in medium. Peptide is added where required at a concentration of 1 µg/ml. For the assay, 10^4 ^{51}Cr-labeled target cells are added to different concentrations of effector cells (final volume of 200 µl) in U-bottom 96-well plates. After a 6 hour incubation period at 37°C, a 0.1 ml aliquot of supernatant is removed from each well and radioactivity is determined in a Micromedic automatic gamma counter. The percent specific lysis is determined by the formula: percent specific release = 100 x (experimental release - spontaneous release)/(maximum release - spontaneous release). To facilitate comparison between separate CTL assays run under the same conditions, % ^{51}Cr release data is expressed as lytic units/10^6 cells. One lytic unit is arbitrarily defined as the number of effector cells required to achieve 30% lysis of 10,000 target cells in a 6 hour ^{51}Cr release assay. To obtain specific lytic units/10^6, the lytic units/10^6 obtained in the absence of peptide is subtracted from the lytic units/10^6 obtained in the presence of peptide. For example, if 30% ^{51}Cr release is obtained at

the effector (E): target (T) ratio of 50:1 (i.e., $5x10^5$ effector cells for 10,000 targets) in the absence of peptide and 5:1 (i.e., $5x10^4$ effector cells for 10,000 targets) in the presence of peptide, the specific lytic units would be: $[(1/50,000)-(1/500,000)] \times 10^6 = 18$ LU.

The results are analyzed to assess the magnitude of the CTL responses of animals injected with the immunogenic CTL/HTL conjugate vaccine preparation. The magnitude and frequency of the response can also be compared to the the CTL response achieved using the CTL epitopes by themselves. Analyses similar to this may be performed to evaluate the immunogenicity of peptide conjugates containing multiple CTL epitopes and/or multiple HTL epitopes. In accordance with these procedures it is found that a CTL response is induced, and concomitantly that an HTL response is induced upon administration of such compositions.

Example 10. Selection of CTL and HTL epitopes for inclusion in a cancer vaccine.

This example illustrates the procedure for the selection of peptide epitopes for vaccine compositions of the invention. The peptides in the composition can be in the form of a nucleic acid sequence, either single or one or more sequences (i.e., minigene) that encodes peptide(s), or may be single and/or polyepitopic peptides.

The following principles are utilized when selecting an array of epitopes for inclusion in a vaccine composition. Each of the following principles is balanced in order to make the selection.

Epitopes are selected which, upon administration, mimic immune responses that have been observed to be correlated with tumor clearance. For example, a vaccine can include 3-4 epitopes that come from at least one TAA. Epitopes from one TAA can be used in combination with epitopes from one or more additional TAAs to produce a vaccine that targets tumors with varying expression patterns of frequently-expressed TAAs as described, e.g., in Example 15.

Epitopes are preferably selected that have a binding affinity (IC50) of 500 nM or less, often 200 nM or less, for an HLA class I molecule, or for a class II molecule, 1000 nM or less.

Sufficient supermotif bearing peptides, or a sufficient array of allele-specific motif bearing peptides, are selected to give broad population coverage. For example, epitopes are selected to provide at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art, can be employed to assess breadth, or redundancy, of population coverage.

When selecting epitopes from cancer-related antigens it is often preferred to select analogs because the patient may have developed tolerance to the native epitope.

When creating a polyepitopic composition, *e.g.* a minigene, it is typically desirable to generate the smallest peptide possible that encompasses the epitopes of interest, although spacers or other flanking sequences can also be incorporated. The principles employed are often similar as those employed when selecting a peptide comprising nested epitopes. Additionally, however, upon determination of the nucleic acid sequence to be provided as a minigene, the peptide sequence encoded thereby is analyzed to determine whether any "junctional epitopes" have been created. A junctional epitope is a potential HLA binding epitope, as predicted, *e.g.*, by motif analysis. Junctional epitopes are generally to be avoided because the recipient may bind to an HLA molecule and generate an immune response to that epitope, which is not present in a native protein sequence.

CTL epitopes for inclusion in vaccine compositions are, for example, selected from those listed in Tables XXVII and XXIII-XXVI. Examples of HTL epitopes that can be included in vaccine compositions are provided in Table XXXI. A vaccine composition comprised of selected peptides, when administered, is safe, efficacious, and elicits an immune response that results in tumor cell killing and reduction of tumor size or mass.

Example 11. Construction of Minigene Multi-Epitope DNA Plasmids

This example provides general guidance for the construction of a minigene expression plasmid. Minigene plasmids may, of course, contain various configurations of CTL and/or HTL epitopes or epitope analogs as described herein. Expression plasmids have been constructed and evaluated as described, for example, in co-pending U.S.S.N. 09/311,784 filed 5/13/99.

A minigene expression plasmid may include multiple CTL and HTL peptide epitopes. In the present example, HLA-A2, -A3, -B7 supermotif-bearing peptide epitopes and HLA-A1 and -A24 motif-bearing peptide epitopes are used in conjunction with DR supermotif-bearing epitopes and/or DR3 epitopes. Preferred epitopes are identified, for example, in Tables XXVII, XXIII-XXVI, and XXXI. HLA class I supermotif or motif-bearing peptide epitopes derived from multiple TAAs are selected such that multiple supermotifs/motifs are represented to ensure broad population coverage. Similarly, HLA class II epitopes are selected from multiple tumor antigens to provide broad population coverage, *i.e.* both HLA DR-1-4-7 supermotif-bearing epitopes and HLA DR-3 motif-bearing epitopes are selected for inclusion in the minigene construct. The selected CTL and HTL epitopes are then incorporated into a minigene for expression in an expression vector.

This example illustrates the methods to be used for construction of such a minigene-bearing expression plasmid. Other expression vectors that may be used for minigene compositions are available and known to those of skill in the art.

The minigene DNA plasmid contains a consensus Kozak sequence and a consensus murine kappa Ig-light chain signal sequence followed by CTL and/or HTL epitopes selected in accordance with principles disclosed herein. The sequence encodes an open reading frame fused to the Myc and His antibody epitope tag coded for by the pcDNA 3.1 Myc-His vector.

Overlapping oligonucleotides, for example eight oligonucleotides, averaging approximately 70 nucleotides in length with 15 nucleotide overlaps, are synthesized and HPLC-purified. The oligonucleotides encode the selected peptide epitopes as well as appropriate linker nucleotides, Kozak sequence, and signal sequence. The final multiepitope minigene is assembled by extending the overlapping oligonucleotides in three sets of reactions using PCR. A Perkin/Elmer 9600 PCR machine is used and a total of 30 cycles are performed using the following conditions: 95°C for 15 sec, annealing temperature (5° below the lowest calculated Tm of each primer pair) for 30 sec, and 72°C for 1 min.

For the first PCR reaction, 5 µg of each of two oligonucleotides are annealed and extended: Oligonucleotides 1+2, 3+4, 5+6, and 7+8 are combined in 100 µl reactions containing *Pfu* polymerase buffer (1x= 10 mM KCL, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-chloride, pH 8.75, 2 mM $MgSO_4$, 0.1% Triton X-100, 100 µg/ml BSA), 0.25 mM each dNTP, and 2.5 U of *Pfu* polymerase. The full-length dimer products are gel-purified, and two reactions containing the product of 1+2 and 3+4, and the product of 5+6

and 7+8 are mixed, annealed, and extended for 10 cycles. Half of the two reactions are then mixed, and 5 cycles of annealing and extension carried out before flanking primers are added to amplify the full length product for 25 additional cycles. The full-length product is gel-purified and cloned into pCR-blunt (Invitrogen) and individual clones are screened by sequencing.

Example 12. The plasmid construct and the degree to which it induces immunogenicity.

The degree to which the plasmid construct prepared using the methodology outlined in Example 11 is able to induce immunogenicity is evaluated through *in vivo* injections into mice and subsequent *in vitro* assessment of CTL and HTL activity, which are analysed using cytotoxicity and proliferation assays, respectively, as detailed *e.g.*, in U.S.S.N. 09/311,784 filed 5/13/99 and Alexander *et al.*, *Immunity* 1:751-761, 1994.

Alternatively, plasmid constructs can be evaluated *in vitro* by testing for epitope presentation by APC following transduction or transfection of the APC with an epitope-expressing nucleic acid construct. Such a study determines "antigenicity" and allows the use of human APC. The assay determines the ability of the epitope to be presented by the APC in a context that is recognized by a T cell by quantifying the density of epitope-HLA class I complexes on the cell surface. Quantitation can be performed by directly measuring the amount of peptide eluted from the APC (*see, e.g.*, Sijts *et al.*, *J. Immunol.* 156:683-692, 1996; Demotz *et al.*, *Nature* 342:682-684, 1989); or the number of peptide-HLA class I complexes can be estimated by measuring the amount of lysis or lymphokine release induced by infected or transfected target cells, and then determining the concentration of peptide necessary to obtained equivalent levels of lysis or lymphokine release (*see, e.g.*, Kageyama *et al.*, *J. Immunol.* 154:567-576, 1995).

To assess the capacity of the pMin minigene construct (*e.g.*, a pMin minigene construct generated as decribed in U.S.S.N. 09/311,784) to induce CTLs *in vivo*, HLA-A11/K^b transgenic mice, for example, are immunized intramuscularly with 100 μg of naked cDNA. As a means of comparing the level of CTLs induced by cDNA immunization, a control group of animals is also immunized with an actual peptide composition that comprises multiple epitopes synthesized as a single polypeptide as they would be encoded by the minigene.

Splenocytes from immunized animals are stimulated twice with each of the respective compositions (peptide epitopes encoded in the minigene or the polyepitopic peptide), then assayed for peptide-specific cytotoxic activity in a ^{51}Cr release assay. The results indicate the magnitude of the CTL response directed against the A3-restricted epitope, thus indicating the *in vivo* immunogenicity of the minigene vaccine and polyepitopic vaccine. It is, therefore, found that the minigene elicits immune responses directed toward the HLA-A3 supermotif peptide epitopes as does the polyepitopic peptide vaccine. A similar analysis is also performed using other HLA-A2 and HLA-B7 transgenic mouse models to assess CTL induction by HLA-A2 and HLA-B7 motif or supermotif epitopes.

To assess the capacity of a class II epitope encoding minigene to induce HTLs *in vivo*, I-A^b restricted mice, for example, are immunized intramuscularly with 100 μg of plasmid DNA. As a means of comparing the level of HTLs induced by DNA immunization, a group of control animals is also immunized with an actual peptide composition emulsified in complete Freund's adjuvant. CD4+ T cells, *i.e.* HTLs, are purified from splenocytes of immunized animals and stimulated with each of the respective compositions

(peptides encoded in the minigene). The HTL response is measured using a ^{3}H-thymidine incorporation proliferation assay, (*see, e.g.*, Alexander et al. Immunity 1:751-761, 1994). The results indicate the magnitude of the HTL response, thus demonstrating the *in vivo* immunogenicity of the minigene.

DNA minigenes, constructed as described in Example 11, may also be evaluated as a vaccine in combination with a boosting agent using a prime boost protocol. The boosting agent may consist of recombinant protein (*e.g.*, Barnett *et al.*, *Aids Res. and Human Retroviruses* 14, Supplement 3:S299-S309, 1998) or recombinant vaccinia, for example, expressing a minigene or DNA encoding the complete protein of interest (*see, e.g.*, Hanke *et al.*, *Vaccine* 16:439-445, 1998; Sedegah *et al.*, *Proc. Natl. Acad. Sci USA* 95:7648-53, 1998; Hanke and McMichael, *Immunol. Letters* 66:177-181, 1999; and Robinson *et al.*, *Nature Med.* 5:526-34, 1999).

For example, the efficacy of the DNA minigene may be evaluated in transgenic mice. In this example, A2.1/K^b transgenic mice are immunized IM with 100 μg of the DNA minigene encoding the immunogenic peptides. After an incubation period (ranging from 3-9 weeks), the mice are boosted IP with 10^7 pfu/mouse of a recombinant vaccinia virus expressing the same sequence encoded by the DNA minigene. Control mice are immunized with 100 μg of DNA or recombinant vaccinia without the minigene sequence, or with DNA encoding the minigene, but without the vaccinia boost. After an additional incubation period of two weeks, splenocytes from the mice are immediately assayed for peptide-specific activity in an ELISPOT assay. Additionally, splenocytes are stimulated *in vitro* with the A2-restricted peptide epitopes encoded in the minigene and recombinant vaccinia, then assayed for peptide-specific activity in an IFN-γ ELISA. It is found that the minigene utilized in a prime-boost mode elicits greater immune responses toward the HLA-A2 supermotif peptides than with DNA alone. Such an analysis is also performed using other HLA-A11 and HLA-B7 transgenic mouse models to assess CTL induction by HLA-A3 and HLA-B7 motif or supermotif epitopes.

Example 13. Peptide Composition for Prophylactic Uses

Vaccine compositions of the present invention are used to prevent cancer in persons who are at risk for developing a tumor. For example, a polyepitopic peptide epitope composition (or a nucleic acid comprising the same) containing multiple CTL and HTL epitopes such as those selected in Examples 9 and/or 10, which are also selected to target greater than 80% of the population, is administered to an individual at risk for a cancer, *e.g.*, breast cancer. The composition is provided as a single polypeptide that encompasses multiple epitopes. The vaccine is administered in an aqueous carrier comprised of Freunds Incomplete Adjuvant. The dose of peptide for the initial immunization is from about 1 to about 50,000 μg, generally 100-5,000 μg, for a 70 kg patient. The initial administration of vaccine is followed by booster dosages at 4 weeks followed by evaluation of the magnitude of the immune response in the patient, by techniques that determine the presence of epitope-specific CTL populations in a PBMC sample. Additional booster doses are administered as required. The composition is found to be both safe and efficacious as a prophylaxis against cancer.

Alternatively, the polyepitopic peptide composition can be administered as a nucleic acid in accordance with methodologies known in the art and disclosed herein.

Example 14. Polyepitopic Vaccine Compositions Derived from Native TAA Sequences

A native TAA polyprotein sequence is screened, preferably using computer algorithms defined for each class I and/or class II supermotif or motif, to identify "relatively short" regions of the polyprotein that comprise multiple epitopes and is preferably less in length than an entire native antigen. This relatively short sequence that contains multiple distinct, even overlapping, epitopes is selected and used to generate a minigene construct. The construct is engineered to express the peptide, which corresponds to the native protein sequence. The "relatively short" peptide is generally less than 1000, 500, or 250 amino acids in length, often less than 100 amino acids in length, preferably less than 75 amino acids in length, and more preferably less than 50 amino acids in length. The protein sequence of the vaccine composition is selected because it has maximal number of epitopes contained within the sequence, *i.e.*, it has a high concentration of epitopes. As noted herein, epitope motifs may be nested or overlapping (*i.e.*, frame shifted relative to one another). For example, with frame shifted overlapping epitopes, two 9-mer epitopes and one 10-mer epitope can be present in a 10 amino acid peptide. Such a vaccine composition is administered for therapeutic or prophylactic purposes.

The vaccine composition will preferably include, for example, three CTL epitopes and at least one HTL epitope from TAAs. This polyepitopic native sequence is administered either as a peptide or as a nucleic acid sequence which encodes the peptide. Alternatively, an analog can be made of this native sequence, whereby one or more of the epitopes comprise substitutions that alter the cross-reactivity and/or binding affinity properties of the polyepitopic peptide.

The embodiment of this example provides for the possibility that an as yet undiscovered aspect of immune system processing will apply to the native nested sequence and thereby facilitate the production of therapeutic or prophylactic immune response-inducing vaccine compositions. Additionally such an embodiment provides for the possibility of motif-bearing epitopes for an HLA makeup that is presently unknown. Furthermore, this embodiment (absent analogs) directs the immune response to multiple peptide sequences that are actually present in native TAAs thus avoiding the need to evaluate any junctional epitopes. Lastly, the embodiment provides an economy of scale when producing nucleic acid vaccine compositions.

Related to this embodiment, computer programs can be derived in accordance with principles in the art, which identify in a target sequence, the greatest number of epitopes per sequence length.

Example 15. Polyepitopic Vaccine Compositions Directed To Multiple Tumors

The HER2/neu peptide epitopes of the present invention are used in conjunction with peptide epitopes from other target tumor antigens to create a vaccine composition that is useful for the treatment of various types of tumors. For example, a set of TAA epitopes can be selected that allows the targeting of most common epithelial tumors (*see, e.g.*, Kawashima *et al.*, *Hum. Immunol.* 59:1-14, 1998). Such a composition includes epitopes from CEA, HER-2/neu, and MAGE2/3, all of which are expressed to appreciable degrees (20-60%) in frequently found tumors such as lung, breast, and gastrointestinal tumors.

The composition can be provided as a single polypeptide that incorporates the multiple epitopes from the various TAAs, or can be administered as a composition comprising one or more discrete epitopes.

Alternatively, the vaccine can be administered as a minigene construct or as dendritic cells which have been loaded with the peptide epitopes *in vitro*.

Targeting multiple tumor antigens is also important to provide coverage of a large fraction of tumors of any particular type. A single TAA is rarely expressed in the majority of tumors of a given type. For example, approximately 50% of breast tumors express CEA, 20% express MAGE3, and 30% express HER-2/neu. Thus, the use of a single antigen for immunotherapy would offer only limited patient coverage. The combination of the three TAAs, however, would address approximately 70% of breast tumors. A vaccine composition comprising epitopes from multiple tumor antigens also reduces the potential for escape mutants due to loss of expression of an individual tumor antigen.

Example 16. Use of peptides to evaluate an immune response

Peptides of the invention may be used to analyze an immune response for the presence of specific CTL or HTL populations directed to a TAA. Such an analysis may be performed using multimeric complexes as described, *e.g.*, by Ogg *et al., Science* 279:2103-2106, 1998 and Greten *et al., Proc. Natl. Acad. Sci. USA* 95:7568-7573, 1998. In the following example, peptides in accordance with the invention are used as a reagent for diagnostic or prognostic purposes, not as an immunogen.

In this example, highly sensitive human leukocyte antigen tetrameric complexes ("tetramers") are used for a cross-sectional analysis of, for example, tumor-associated antigen HLA-A*0201-specific CTL frequencies from HLA A*0201-positive individuals at different stages of disease or following immunization using a TAA peptide containing an A*0201 motif. Tetrameric complexes are synthesized as described (Musey *et al., N. Engl. J. Med.* 337:1267, 1997). Briefly, purified HLA heavy chain (A*0201 in this example) and β2-microglobulin are synthesized by means of a prokaryotic expression system. The heavy chain is modified by deletion of the transmembrane-cytosolic tail and COOH-terminal addition of a sequence containing a BirA enzymatic biotinylation site. The heavy chain, β2-microglobulin, and peptide are refolded by dilution. The 45-kD refolded product is isolated by fast protein liquid chromatography and then biotinylated by BirA in the presence of biotin (Sigma, St. Louis, Missouri), adenosine 5'triphosphate and magnesium. Streptavidin-phycoerythrin conjugate is added in a 1:4 molar ratio, and the tetrameric product is concentrated to 1 mg/ml. The resulting product is referred to as tetramer-phycoerythrin.

For the analysis of patient blood samples, approximately one million PBMCs are centrifuged at 300g for 5 minutes and resuspended in 50 μl of cold phosphate-buffered saline. Tri-color analysis is performed with the tetramer-phycoerythrin, along with anti-CD8-Tricolor, and anti-CD38. The PBMCs are incubated with tetramer and antibodies on ice for 30 to 60 min and then washed twice before formaldehyde fixation. Gates are applied to contain >99.98% of control samples. Controls for the tetramers include both A*0201-negative individuals and A*0201-positive uninfected donors. The percentage of cells stained with the tetramer is then determined by flow cytometry. The results indicate the number of cells in the PBMC sample that contain epitope-restricted CTLs, thereby readily indicating the extent of immune response to the TAA epitope, and thus the stage of tumor progression or exposure to a vaccine that elicits a protective or therapeutic response.

Example 17. Use of Peptide Epitopes to Evaluate Recall Responses

The peptide epitopes of the invention are used as reagents to evaluate T cell responses, such as acute or recall responses, in patients. Such an analysis may be performed on patients who are in remission, have a tumor, or who have been vaccinated with a TAA vaccine.

For example, the class I restricted CTL response of persons who have been vaccinated may be analyzed. The vaccine may be any TAA vaccine. PBMC are collected from vaccinated individuals and HLA typed. Appropriate peptide epitopes of the invention that, optimally, bear supermotifs to provide cross-reactivity with multiple HLA supertype family members, are then used for analysis of samples derived from individuals who bear that HLA type.

PBMC from vaccinated individuals are separated on Ficoll-Histopaque density gradients (Sigma Chemical Co., St. Louis, MO), washed three times in HBSS (GIBCO Laboratories), resuspended in RPMI-1640 (GIBCO Laboratories) supplemented with L-glutamine (2mM), penicillin (50U/ml), streptomycin (50 μg/ml), and Hepes (10mM) containing 10% heat-inactivated human AB serum (complete RPMI) and plated using microculture formats. A synthetic peptide comprising an epitope of the invention is added at 10 μg/ml to each well and HBV core 128-140 epitope is added at 1 μg/ml to each well as a source of T cell help during the first week of stimulation.

In the microculture format, 4 x 10^5 PBMC are stimulated with peptide in 8 replicate cultures in 96-well round bottom plate in 100 μl/well of complete RPMI. On days 3 and 10, 100 μl of complete RPMI and 20 U/ml final concentration of rIL-2 are added to each well. On day 7 the cultures are transferred into a 96-well flat-bottom plate and restimulated with peptide, rIL-2 and 10^5 irradiated (3,000 rad) autologous feeder cells. The cultures are tested for cytotoxic activity on day 14. A positive CTL response requires two or more of the eight replicate cultures to display greater than 10% specific ^{51}Cr release, based on comparison with uninfected control subjects as previously described (Rehermann, *et al., Nature Med.* 2:1104,1108, 1996; Rehermann *et al., J. Clin. Invest.* 97:1655-1665, 1996; and Rehermann *et al. J. Clin. Invest.* 98:1432-1440, 1996).

Target cell lines are autologous and allogeneic EBV-transformed B-LCL that are either purchased from the American Society for Histocompatibility and Immunogenetics (ASHI, Boston, MA) or established from the pool of patients as described (Guilhot, *et al. J. Virol. 66*:2670-2678, 1992).

Cytotoxicity assays are performed in the following manner. Target cells consist of either allogeneic HLA-matched or autologous EBV-transformed B lymphoblastoid cell line that are incubated overnight with the synthetic peptide epitope of the invention at 10 μM, and labeled with 100 μCi of ^{51}Cr (Amersham Corp., Arlington Heights, IL) for 1 hour after which they are washed four times with HBSS.

Cytolytic activity is determined in a standard 4 hour, split-well ^{51}Cr release assay using U-bottomed 96 well plates containing 3,000 targets/well. Stimulated PBMC are tested at effector/target (E/T) ratios of 20-50:1 on day 14. Percent cytotoxicity is determined from the formula: 100 x [(experimental release-spontaneous release)/maximum release-spontaneous release)]. Maximum release is determined by lysis of targets by detergent (2% Triton X-100; Sigma Chemical Co., St. Louis, MO). Spontaneous release is <25% of maximum release for all experiments.

The results of such an analysis indicate the extent to which HLA-restricted CTL populations have been stimulated by previous exposure to the TAA or TAA vaccine.

The class II restricted HTL responses may also be analyzed. Purified PBMC are cultured in a 96-well flat bottom plate at a density of $1.5x10^5$ cells/well and are stimulated with 10 μg/ml synthetic peptide, whole antigen, or PHA. Cells are routinely plated in replicates of 4-6 wells for each condition. After seven days of culture, the medium is removed and replaced with fresh medium containing 10U/ml IL-2. Two days later, 1 μCi ^{3}H-thymidine is added to each well and incubation is continued for an additional 18 hours. Cellular DNA is then harvested on glass fiber mats and analyzed for ^{3}H-thymidine incorporation. Antigen-specific T cell proliferation is calculated as the ratio of ^{3}H-thymidine incorporation in the presence of antigen divided by the ^{3}H-thymidine incorporation in the absence of antigen.

Example 18. Induction Of Specific CTL Response In Humans

A human clinical trial for an immunogenic composition comprising CTL and HTL epitopes of the invention is set up as an IND Phase I, dose escalation study. Such a trial is designed, for example, as follows:

A total of about 27 subjects are enrolled and divided into 3 groups:

Group I: 3 subjects are injected with placebo and 6 subjects are injected with 5 μg of peptide composition;

Group II: 3 subjects are injected with placebo and 6 subjects are injected with 50 μg peptide composition;

Group III: 3 subjects are injected with placebo and 6 subjects are injected with 500 μg of peptide composition.

After 4 weeks following the first injection, all subjects receive a booster inoculation at the same dosage. Additional booster inoculations can be administered on the same schedule.

The endpoints measured in this study relate to the safety and tolerability of the peptide composition as well as its immunogenicity. Cellular immune responses to the peptide composition are an index of the intrinsic activity of the peptide composition, and can therefore be viewed as a measure of biological efficacy. The following summarize the clinical and laboratory data that relate to safety and efficacy endpoints.

Safety: The incidence of adverse events is monitored in the placebo and drug treatment group and assessed in terms of degree and reversibility.

Evaluation of Vaccine Efficacy: For evaluation of vaccine efficacy, subjects are bled before and after injection. Peripheral blood mononuclear cells are isolated from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation, aliquoted in freezing media and stored frozen. Samples are assayed for CTL and HTL activity.

The vaccine is found to be both safe and efficacious.

Example 19. Therapeutic Use in Cancer Patients

Evaluation of vaccine compositions are performed to validate the efficacy of the CTL-HTL peptide compositions in cancer patients. The main objectives of the trials are to determine an effective dose and regimen for inducing CTLs in cancer patients, to establish the safety of inducing a CTL and HTL response in these patients, and to see to what extent activation of CTLs improves the clinical picture of cancer

patients, as manifested by a reduction in tumor cell numbers. Such a study is designed, for example, as follows:

The studies are performed in multiple centers. The trial design is an open-label, uncontrolled, dose escalation protocol wherein the peptide composition is administered as a single dose followed six weeks later by a single booster shot of the same dose. The dosages are 50, 500 and 5,000 micrograms per injection. Drug-associated adverse effects (severity and reversibility) are recorded.

There are three patient groupings. The first group is injected with 50 micrograms of the peptide composition and the second and third groups with 500 and 5,000 micrograms of peptide composition, respectively. The patients within each group range in age from 21-65, include both males and females (unless the tumor is sex-specific, *e.g.*, breast or prostate cancer), and represent diverse ethnic backgrounds.

Example 20. Induction of CTL Responses Using a Prime Boost Protocol

A prime boost protocol similar in its underlying principle to that used to evaluate the efficacy of a DNA vaccine in transgenic mice, which was described in Example 12, can also be used for the administration of the vaccine to humans. Such a vaccine regimen may include an initial administration of, for example, naked DNA followed by a boost using recombinant virus encoding the vaccine, or recombinant protein/polypeptide or a peptide mixture administered in an adjuvant.

For example, the initial immunization can be performed using an expression vector, such as that constructed in Example 11, in the form of naked nucleic acid administered IM (or SC or ID) in the amounts of 0.5-5 mg at multiple sites. The nucleic acid (0.1 to 1000 μg) can also be administered using a gene gun. Following an incubation period of 3-4 weeks, a booster dose is then administered. The booster can be recombinant fowlpox virus administered at a dose of $5\text{-}10^7$ to $5\text{x}10^9$ pfu. An alternative recombinant virus, such as an MVA, canarypox, adenovirus, or adeno-associated virus, can also be used for the booster, or the polyepitopic protein or a mixture of the peptides can be administered. For evaluation of vaccine efficacy, patient blood samples will be obtained before immunization as well as at intervals following administration of the initial vaccine and booster doses of the vaccine. Peripheral blood mononuclear cells are isolated from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation, aliquoted in freezing media and stored frozen. Samples are assayed for CTL and HTL activity.

Analysis of the results will indicate that a magnitude of response sufficient to achieve protective immunity against cancer is generated.

Example 21. Administration of Vaccine Compositions Using Dendritic Cells

Vaccines comprising peptide epitopes of the invention can be administered using antigen-presenting cells, or "professional" APCs such as dendritic cells. In this example, the peptide-pulsed DC are administered to a patient to stimulate a CTL response *in vivo*. In this method, dendritic cells are isolated, expanded, and pulsed with a vaccine comprising peptide CTL and HTL epitopes of the invention. The dendritic cells are infused back into the patient to elicit CTL and HTL responses *in vivo*. The induced lymphocytes then destroy or facilitate destruction of the specific target tumor cells that bear the proteins from which the epitopes in the vaccine are derived.

For example, a cocktail of epitope-bearing peptides is administered *ex vivo* to PBMC, or isolated DC therefrom, from the patient's blood. A pharmaceutical to facilitate harvesting of DC can be used, such as Progenipoietin™ (Monsanto, St. Louis, MO) or GM-CSF/IL-4. After pulsing the DC with peptides and prior to reinfusion into patients, the DC are washed to remove unbound peptides.

As appreciated clinically, and readily determined by one of skill based on clinical outcomes, the number of dendritic cells reinfused into the patient can vary (*see, e.g., Nature Med.* 4:328, 1998; *Nature Med.* 2:52, 1996 and *Prostate* 32:272, 1997). Although 2-50 x 10^6 dendritic cells per patient are typically administered, larger number of dendritic cells, such as 10^7 or 10^8 can also be provided. Such cell populations typically contain between 50-90% dendritic cells.

In some embodiments, peptide-loaded PBMC are injected into patients without purification of the DC. For example, PBMC containing DC generated after treatment with an agent such as Progenipoietin™ are injected into patients without purification of the DC. The total number of PBMC that are administered often ranges from 10^8 to 10^{10}. Generally, the cell doses injected into patients is based on the percentage of DC in the blood of each patient, as determined, for example, by immunofluorescence analysis with specific anti-DC antibodies. Thus, for example, if Progenipoietin™ mobilizes 2% DC in the peripheral blood of a given patient, and that patient is to receive 5 x 10^6 DC, then the patient will be injected with a total of 2.5 x 10^8 peptide-loaded PBMC. The percent DC mobilized by an agent such as Progenipoietin™ is typically estimated to be between 2-10%, but can vary as appreciated by one of skill in the art.

Ex vivo activation of CTL/HTL responses

Alternatively, *ex vivo* CTL or HTL responses to a particular tumor-associated antigen can be induced by incubating in tissue culture the patient's, or genetically compatible, CTL or HTL precursor cells together with a source of antigen-presenting cells (APC), such as dendritic cells, and the appropriate immunogenic peptides. After an appropriate incubation time (typically about 7-28 days), in which the precursor cells are activated and expanded into effector cells, the cells are infused back into the patient, where they will destroy (CTL) or facilitate destruction (HTL) of their specific target cells, *i.e.*, tumor cells.

Example 22. Alternative Method of Identifying Motif-Bearing Peptides

Another way of identifying motif-bearing peptides is to elute them from cells bearing defined MHC molecules. For example, EBV transformed B cell lines used for tissue typing, have been extensively characterized to determine which HLA molecules they express. In certain cases these cells express only a single type of HLA molecule. These cells can then be infected with a pathogenic organism or transfected with nucleic acids that express the tumor antigen of interest. Thereafter, peptides produced by endogenous antigen processing of peptides produced consequent to infection (or as a result of transfection) will bind to HLA molecules within the cell and be transported and displayed on the cell surface.

The peptides are then eluted from the HLA molecules by exposure to mild acid conditions and their amino acid sequence determined, *e.g.*, by mass spectral analysis (*e.g.*, Kubo *et al.*, *J. Immunol.* 152:3913, 1994). Because, as disclosed herein, the majority of peptides that bind a particular HLA molecule are motif-bearing, this is an alternative modality for obtaining the motif-bearing peptides correlated with the particular HLA molecule expressed on the cell.

Alternatively, cell lines that do not express any endogenous HLA molecules can be transfected with an expression construct encoding a single HLA allele. These cells may then be used as described, *i.e.*, they may be infected with a pathogenic organism or transfected with nucleic acid encoding an antigen of interest to isolate peptides corresponding to the pathogen or antigen of interest that have been presented on the cell surface. Peptides obtained from such an analysis will bear motif(s) that correspond to binding to the single HLA allele that is expressed in the cell.

As appreciated by one in the art, one can perform a similar analysis on a cell bearing more than one HLA allele and subsequently determine peptides specific for each HLA allele expressed. Moreover, one of skill would also recognize that means other than infection or transfection, such as loading with a protein antigen, can be used to provide a source of antigen to the cell.

The above examples are provided to illustrate the invention but not to limit its scope. For example, the human terminology for the Major Histocompatibility Complex, namely HLA, is used throughout this document. It is to be appreciated that these principles can be extended to other species as well. Thus, other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, patents, and patent application cited herein are hereby incorporated by reference for all purposes.

TABLE I

SUPERMOTIFS	POSITION 2 (Primary Anchor)	POSITION 3 (Primary Anchor)	POSITION C Terminus (Primary Anchor)
A1	**T, I,** *L, V, M, S*		**F, W, Y**
A2	**L, I, V, M,** *A, T, Q*		**I, V,** *M, A, T, L*
A3	**V, S, M, A,** *T, L, I*		**R,K**
A24	**Y, F,** *W, I, V, L, M, T*		**F, I,** *Y, W, L, M*
B7	**P**		**V, I, L, F,** *M, W, Y, A*
B27	**R, H, K**		**F, Y, L,** *W, M, I, V, A*
B44	**E,** *D*		**F, W, L, I, M, V, A**
B58	**A, T, S**		**F, W, Y,** *L, I, V, M, A*
B62	**Q, L,** *I, V, M, P*		**F, W, Y,** *M, I, V, L, A*
MOTIFS			
A1	**T, S, M**		**Y**
A1		**D, E,** *A, S*	**Y**
A2.1	**L, M,** *V, Q, I, A, T*		**V,** *L, I, M, A, T*
A3	**L, M, V, I, S, A, T, F,** *C, G, D*		**K, Y, R,** *H, F, A*
A11	**V, T, M, L, I, S, A, G, N,** *C, D, F*		**K,** *R, Y, H*
A24	**Y, F, W,** *M*		**F, L, I, W**
A*3101	**M, V, T,** *A ,L, I, S*		**R,** *K*
A*3301	**M, V, A, L, F,** *I, S, T*		**R, K**
A*6801	**A, V, T,** *M, S, L, I*		**R, K**
B*0702	**P**		**L, M, F,** *W, Y, A, I, V*
B*3501	**P**		**L, M, F, W, Y,** *I, V, A*
B51	**P**		**L, I, V, F,** *W, Y, A, M*
B*5301	**P**		**I, M, F, W, Y,** *A, L, V*
B*5401	**P**		**A, T, I, V,** *L, M, F, W, Y*

Bolded residues are preferred, italicized residues are less preferred: A peptide is considered motif-bearing if it has primary anchors at each primary anchor position for a motif or supermotif as specified in the above table.

TABLE Ia

SUPERMOTIFS	POSITION	POSITION	POSITION
	2 (Primary Anchor)	3 (Primary Anchor)	C Terminus (Primary Anchor)
A1	**T, I,** *L, V, M, S*		**F, W, Y**
A2	**V,** *Q, A, T*		**I, V,** *L, M, A, T*
A3	**V, S, M, A,** *T, L, I*		**R, K**
A24	**Y, F,** *W, I, V, L, M, T*		**F, I,** *Y, W, L, M*
B7	**P**		**V, I, L, F,** *M, W, Y, A*
B27	**R, H, K**		**F, Y, L,** *W, M, I, V, A*
B58	**A, T, S**		**F, W, Y,** *L, I, V, M, A*
B62	**Q, L,** *I, V, M, P*		**F, W, Y,** *M, I, V, L, A*
MOTIFS			
A1	**T, S, M**		**Y**
A1		**D, E,** *A, S*	**Y**
A2.1	*V, Q, A, T**		**V,** *L, I, M, A, T*
A3.2	**L, M, V, I, S, A, T, F,** *C, G, D*		**K, Y, R,** *H, F, A*
A11	**V, T, M, L, I, S, A, G, N,** *C, D, F*		**K,** *R, H, Y*
A24	**Y ,F, W**		**F, L, I, W**

*If 2 is V, or Q, the C-term is not L

Bolded residues are preferred, italicized residues are less preferred: A peptide is considered motif-bearing if it has primary anchors at each primary anchor position for a motif or supermotif as specified in the above table.

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TABLE II

		POSITION 1	2	3	4	5	6	7	8	C-terminus
SUPERMOTIFS										
A1			1° Anchor T,I,*L,V,M,S*							1° Anchor F,W,Y
A2			1° Anchor L,I,V,M,*A, T,Q*							1° Anchor L,I,V,M,A,T
A3	preferred		1° Anchor V,S,M,A,*T, L,I*	Y,F,W, (4/5)			Y,F,W, (3/5)	Y,F,W, (4/5)	P, (4/5)	1°Anchor R,K
	deleterious	D,E (3/5); P, (5/5)		D,E, (4/5)						
A24			1° Anchor Y,F,*W,I,V, L,M,T*							1° Anchor F,I,*Y,W,L,M*
B7	preferred	F,W,Y (5/5) L,I,V,M, (3/5)	1°Anchor P	F,W,Y (4/5)					F,W,Y, (3/5)	1°Anchor V,I,L,F,*M,W,Y,A*
	deleterious	D,E (3/5); P(5/5); G(4/5); A(3/5); Q,N, (3/5)				D,E, (3/5)	G, (4/5)	Q,N, (4/5)	D,E, (4/5)	
B27			1° Anchor R,H,K							1° Anchor F,Y,L,*W,M,V,A*
B44			1° Anchor E,*D*							1° Anchor F,W,Y,L,I,M,V,A
B58			1° Anchor A,T,S							1° Anchor F,W,Y,*L,I,V,M,A*
B62			1° Anchor Q,L,*I,V,M, P*							1° Anchor F,W,Y,*M,I,V,L,A*

		POSITION								
		1	2	3	4	5	6	7	8	C-terminus
MOTIFS										
A1 9-mer	preferred	G,F,Y,W,	1°Anchor S,T,M,	D,E,A,	Y,F,W,		P,	D,E,Q,N,	Y,F,W,	1°Anchor Y
	deleterious	D,E,		R,H,K,L,I,V M,P,	A,	G,	A,			
A1 9-mer	preferred	G,R,H,K	A,S,T,C,L,I V,M,	1°Anchor D,E,*A,S*	G,S,T,C,		A,S,T,C,	L,I,V,M,	D,E,	1°Anchor Y
	deleterious	A	R,H,K,D,E, P,Y,F,W,		D,E,	P,Q,N,	R,H,K,	P,G,	G,P,	

		POSITION									
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
A1 10-mer	peferred	Y,F,W,	1°Anchor S,T,M	D,E,A,Q,N,	A,	Y,F,W,Q,N,		P,A,S,T,C,	G,D,E,	P,	1°Anchor Y
	deleterious	G,P,		R,H,K,G,L,I V,M,	D,E,	R,H,K,	Q,N,A	R,H,K,Y,F, W,	R,H,K,	A	
A1 10-mer	preferred	Y,F,W,	S,T,C,L,I,V M,	1°Anchor D,E,*A,S*	A,	Y,F,W,		P,G,	G,	Y,F,W,	1°Anchor Y
	deleterious	R,H,K,	R,H,K,D,E, P,Y,F,W,			P,	G,		P,R,H,K,	Q,N,	
A2.1 9-mer	preferred	Y,F,W,	1°Anchor L,M,*I,V,Q, A,T*	Y,F,W,	S,T,C,	Y,F,W,		A,	P	1°Anchor V,*L,I,M,A,T*	
	deleterious	D,E,P,		D,E,R,K,H			R,K,H	D,E,R,K,H			
A2.1 10-mer	preferred	A,Y,F,W,	1°Anchor L,M,*I,V,Q, A,T*	L,V,I,M,	G,		G,		F,Y,W,L, V,I,M,		1°Anchor V,*L,I,M,A,T*
	deleterious	D,E,P,		D,E,	R,K,H,A,	P,		R,K,H,	D,E,R,K, H,	R,K,H,	

		POSITION									
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
A3	preferred	R,H,K,	1°Anchor L,M,V,I,S, A,T,F,*C,G D*	Y,F,W,	P,R,H,K,Y, F,W,	A,	Y,F,W,		P,	1°Anchor K,Y,R,*H,F,A*	
	deleterious	D,E,P,		D,E							
A11	preferred	A,	1°Anchor V,T,L,M,I, S,A,G,N,*C, D,F*	Y,F,W,	Y,FW,	A,	Y,F,W,	Y,FW,	P,	1°Anchor K,,*RY,H*	
	deleterious	D,E,P,						A	G,		
A24 9-mer	preferred	Y,F,W,R,H,K,	1°Anchor Y,F,W,*M*		S,T,C			Y,F,W,	Y,F,W,	1°Anchor F,L,I,W	
	deleterious	D,E,G,		D,E,	G,	Q,N,P,	D,E,R,H,K,	G,	A,Q,N,		
A24 10-mer	preferred		1°Anchor Y,F,W,*M*		P,	Y,F,W,P,		P,			1°Anchor F,L,I,W
	deleterious			G,D,E	Q,N	R,H,K	D,E	A	Q,N,	D,E,A,	
A3101	preferred	R,H,K,	1°Anchor M,V,T,*A,L, I,S*	Y,F,W,	P,		Y,F,W,	Y,F,W,	A,P,	1°Anchor R,*K*	
	deleterious	D,E,P,		D,E,		A,D,E,	D,E,	D,E,	D,E,		

		POSITION									
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
A3301	preferred		1°Anchor M,V,A,L,F, *I,S,T*	Y,F,W				A,Y,F,W		1°Anchor R,K	
	deleterious	G,P		D,E							
A6801	preferred	Y,F,W,S,T,C,	1°Anchor A,V,T,*M,S, L,I*			Y,F,W,L,I, V,M		Y,F,W,	P,	1°Anchor R,K	
	deleterious	G,P,		D,E,G,		R,H,K,			A,		
B0702	preferred	R,H,K,F,W,Y,	1°Anchor P	R,H,K,		R,H,K,	R,H,K,	R,H,K,	P,A,	1°Anchor L,M,F,*W,Y,A, I,V*	
	deleterious	D,E,Q,N,P,		D,E,P,	D,E,	D,E,	G,D,E,	Q,N,	D,E,		
B3501	preferred	F,W,Y,L,I,V,M,	1°Anchor P	F,W,Y,				F,W,Y,		1°Anchor L,M,F,W,Y,*I, V,A*	
	deleterious	A,G,P,				G,	G,				

		POSITION								
		1	2	3	4	5	6	7	8	9 or C-terminus	C-terminus
B51	preferred	L,I,V,M,F,W,Y,	1°Anchor P	F,W,Y,	S,T,C,	F,W,Y,		G,	F,W,Y,	1°Anchor L,I,V,F,*W, Y,A,M*	
	deleterious	A,G,P,D,E,R,H,K, S,T,C,				D,E,	G,	D,E,Q,N,	G,D,E,		
B5301	preferred	L,I,V,M,F,W,Y,	1°Anchor P	F,W,Y,	S,T,C,	F,W,Y,		L,I,V,M,F, W,Y,	F,W,Y,	1°Anchor I,M,F,W,Y, *A,L,V*	
	deleterious	A,G,P,Q,N,					G,	R,H,K,Q,N,	D,E,		
B5401	preferred	F,W,Y,	1°Anchor P	F,W,Y,L,I,V M,		L,I,V,M,		A,L,I,V,M,	F,W,Y,A,P,	1°Anchor A,T,I,V,*L, M,F,W,Y*	
	deleterious	G,P,Q,N,D,E,		G,D,E,S,T,C,		R,H,K,D,E,	D,E,	Q,N,D,G,E,	D,E,		

Italicized residues indicate less preferred or "tolerated" residues.
The information in Table II is specific for 9-mers unless otherwise specified.
Secondary anchor specificities are designated for each position independently.

Table III

		POSITION								
MOTIFS		1° anchor 1	2	3	4	5	1° anchor 6	7	8	9
DR4	preferred	F, M, Y, *L, I, V, W,*	M,	T,		I,	V, S, T, *C, P, A, L, I, M,*	M, H,		M, H
	deleterious				W,			R,		W, D, E
DR1	preferred	M, F, *L, I, V, W, Y,*			P, A, M, Q,		V, M, A, T, *S, P, L, I, C,*	M,		A, V, M
	deleterious		C	C, H	F, D	C, W, D		G, D, E,	D	
DR7	preferred	M, F, *L, I, V, W, Y,*	M,	W,	A,		I, V, M, S, A, *C, T, P, L,*	M,		I, V
	deleterious		C,		G,			G, R, D,	N	G
DR Supermotif		M, F, *L, I, V, W, Y,*					V, M, S, T, A, *C, P, L, I,*			

DR3 MOTIFS	1° anchor 1	2	3	1° anchor 4	5	1° anchor 6
motif a preferred	L, I, V, M, F, Y,			D		
motif b preferred	L, I, V, M, F, A, Y,			D, N, Q, E, S, T		K, R, H

Italicized residues indicate less preferred or "tolerated" residues. Secondary anchor specificities are designated for each position independently.

Table IV: HLA Class I Standard Peptide Binding Affinity.

ALLELE	STANDARD PEPTIDE	SEQUENCE (SEQ ID NO:)	STANDARD BINDING AFFINITY (nM)
A*0101	944.02	YLEPAIAKY	25
A*0201	941.01	FLPSDYFPSV	5.0
A*0202	941.01	FLPSDYFPSV	4.3
A*0203	941.01	FLPSDYFPSV	10
A*0205	941.01	FLPSDYFPSV	4.3
A*0206	941.01	FLPSDYFPSV	3.7
A*0207	941.01	FLPSDYFPSV	23
A*6802	1072.34	YVIKVSARV	8.0
A*0301	941.12	KVFPYALINK	11
A*1101	940.06	AVDLYHFLK	6.0
A*3101	941.12	KVFPYALINK	18
A*3301	1083.02	STLPETYVVRR	29
A*6801	941.12	KVFPYALINK	8.0
A*2402	979.02	AYIDNYNKF	12
B*0702	1075.23	APRTLVYLL	5.5
B*3501	1021.05	FPFKYAAAF	7.2
B51	1021.05	FPFKYAAAF	5.5
B*5301	1021.05	FPFKYAAAF	9.3
B*5401	1021.05	FPFKYAAAF	10

Table V. HLA Class II Standard Peptide Binding Affinity.

Allele	Nomenclature	Standard Peptide	Sequence (SEQ ID NO:)	Binding Affinity (nM)
DRB1*0101	DR1	515.01	PKYVKQNTLKLAT	5.0
DRB1*0301	DR3	829.02	YKTIAFDEEARR	300
DRB1*0401	DR4w4	515.01	PKYVKQNTLKLAT	45
DRB1*0404	DR4w14	717.01	YARFQSQTTLKQKT	50
DRB1*0405	DR4w15	717.01	YARFQSQTTLKQKT	38
DRB1*0701	DR7	553.01	QYIKANSKFIGITE	25
DRB1*0802	DR8w2	553.01	QYIKANSKFIGITE	49
DRB1*0803	DR8w3	553.01	QYIKANSKFIGITE	1600
DRB1*0901	DR9	553.01	QYIKANSKFIGITE	75
DRB1*1101	DR5w11	553.01	QYIKANSKFIGITE	20
DRB1*1201	DR5w12	1200.05	EALIHQLKINPYVLS	298
DRB1*1302	DR6w19	650.22	QYIKANAKFIGITE	3.5
DRB1*1501	DR2w2β1	507.02	GRTQDENPVVHFFKNIV TPRTPPP	9.1
DRB3*0101	DR52a	511	NGQIGNDPNRDIL	470
DRB4*0101	DRw53	717.01	YARFQSQTTLKQKT	58
DRB5*0101	DR2w2β2	553.01	QYIKANSKFIGITE	20

Table VI

HLA-supertype	Allelle-specific HLA-supertype members	
	Verified[a]	Predicted[b]
A1	A*0101, A*2501, A*2601, A*2602, A*3201	A*0102, A*2604, A*3601, A*4301, A*8001
A2	A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*0209, A*0214, A*6802, A*6901	A*0208, A*0210, A*0211, A*0212, A*0213
A3	A*0301, A*1101, A*3101, A*3301, A*6801	A*0302, A*1102, A*2603, A*3302, A*3303, A*3401, A*3402, A*6601, A*6602, A*7401
A24	A*2301, A*2402, A*3001	A*2403, A*2404, A*3002, A*3003
B7	B*0702, B*0703, B*0704, B*0705, B*1508, B*3501, B*3502, B*3503, B*3503, B*3504, B*3505, B*3506, B*3507, B*3508, B*5101, B*5102, B*5103, B*5104, B*5105, B*5301, B*5401, B*5501, B*5502, B*5601, B*5602, B*6701, B*7801	B*1511, B*4201, B*5901
B27	B*1401, B*1402, B*1509, B*2702, B*2703, B*2704, B*2705, B*2706, B*3801, B*3901, B*3902, B*7301	B*2701, B*2707, B*2708, B*3802, B*3903, B*3904, B*3905, B*4801, B*4802, B*1510, B*1518, B*1503
B44	B*1801, B*1802, B*3701, B*4402, B*4403, B*4404, B*4001, B*4002, B*4006	B*4101, B*4501, B*4701, B*4901, B*5001
B58	B*5701, B*5702, B*5801, B*5802, B*1516, B*1517	
B62	B*1501, B*1502, B*1513, B*5201	B*1301, B*1302, B*1504, B*1505, B*1506, B*1507, B*1515, B*1520, B*1521, B*1512, B*1514, B*1510

a. Verified alleles include alleles whose specificity has been determined by pool sequencing analysis, peptide binding assays, or by analysis of the sequences of CTL epitopes.

b. Predicted alleles are alleles whose specificity is predicted on the basis of B and F pocket structure to overlap with the supertype specificity.

Table VII
HER2/NEU A01 Supermotif Peptides with Binding Data

Position	No. of Amino Acids	A*0101
66	8	
272	8	
732	8	
899	8	
296	8	0.1000
916	8	-0.0021
1241	8	0.0030
166	8	
369	8	
76	8	
434	8	
828	8	-0.0021
945	8	
418	8	
1023	8	
2	8	
607	8	
402	8	-0.0021
1016	8	
101	8	
479	8	
664	8	
724	8	
911	8	
1024	8	
1180	8	
603	8	
796	8	
952	8	
357	8	
892	8	
962	8	
997	8	
818	8	
906	8	
165	9	
356	9	
478	9	
910	9	
104	9	0.1800
401	9	0.0430
1131	9	0.1300
100	9	
373	9	
1023	9	
444	9	
513	9	
42	9	9.1000
546	9	0.0050
795	9	0.0024

Table VII
HER2/NEU A01 Supermotif Peptides with Binding Data

Position	No. of Amino Acids	A*0101
869	9	7.6000
1119	9	0.0017
271	9	
663	9	
1179	9	
295	9	0.0042
978	9	
197	9	
281	9	0.0028
773	9	0.0400
915	9	0.0011
417	9	
608	9	
293	9	0.0550
727	9	0.0011
997	9	0.0290
1213	9	0.0430
525	10	
406	10	
443	10	
899	10	2.7000
1239	10	0.0630
467	10	
960	10	
154	10	0.0300
64	10	
270	10	
391	10	
607	10	
662	10	
890	10	
160	10	
473	10	
816	10	
265	10	0.0015
402	10	1.1000
868	10	1.3000
914	10	0.0082
1130	10	0.0072
355	10	
722	10	
909	10	
904	10	
950	10	
55	10	0.0180
545	10	0.0015
772	10	1.1000
826	10	0.3000
249	10	
372	10	

Table VII
HER2/NEU A01 Supermotif Peptides with Binding Data

Position	No. of Amino Acids	A*0101
1077	10	
280	10	0.1800
334	10	0.0016
601	10	0.0010
1213	10	5.5000
40	11	0.2800
401	11	0.4400
1102	11	0.0160
405	11	
98	11	
466	11	
661	11	
442	11	
73	11	
153	11	
725	11	
476	11	
54	11	
793	11	
1117	11	
281	11	
959	11	
1013	11	0.0027
854	11	
976	11	
195	11	
1213	11	
293	11	0.1900

Table VIII
HER2/NEU A02 Supermotif with Binding Data

Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
1094	8					
1094	10					
4	8					
4	9					
4	10	0.0010				
4	11					
1203	10					
1159	8					
1159	9	0.0001				
20	8					
20	10					
751	9					
113	11					
5	8					
5	9	0.0310				
5	10	0.0360	0.0022	0.8600	0.0019	0.0160
5	11					
890	11					
466	9	0.0210				
14	8					
14	10	0.0001				
270	9	0.0001				
705	8					
705	10	0.0007				
705	11					
710	9					
710	11					
1165	8					
1165	9					
1190	8					
1190	9					
115	9	0.0004				
355	11					
657	8					
657	9	0.0007				
657	10	0.0002				
657	11					
587	11					
224	8					
224	10					
338	8					
338	10	0.0011				
255	9					
789	8					
789	9	0.0340				
789	10					
826	8					
826	11					
623	9					
567	8					

Table VIII
HER2/NEU A02 Supermotif with Binding Data

Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
567	9					
212	8					
212	10					
53	8					
53	10					
53	11					
244	10					
244	11					
26	8					
26	10					
630	8					
947	8					
947	9					
947	10					
596	9	0.0004				
634	11					
540	8					
540	11					
504	11					
528	8					
295	10	0.0001				
871	9					
171	9	0.0002				
171	10					
171	11					
76	9	0.0001				
76	10	0.0001				
76	11					
845	8					
845	9	0.0002				
636	9					
1089	10	0.0001				
993	8					
993	11					
933	9	0.0002				
821	9	0.0002				
821	10					
421	8					
421	10	0.0003				
421	11					
1016	9	0.0002				
1016	10	0.0002				
1013	8					
30	8					
1224	9					
483	10					
483	11					
165	8					
1183	8					
1183	9	0.0002				

Table VIII
HER2/NEU A02 Supermotif with Binding Data

Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
1084	9					
1084	11					
307	8					
307	11					
838	9	0.0002				
838	10					
838	11					
904	8					
904	9	0.0002				
950	11					
580	9					
1069	8					
770	8					
766	8					
766	9					
766	10					
147	8					
147	9	0.0001				
405	8					
405	10					
2	10	0.0001				
2	11					
460	8					
460	9	0.0004				
265	8					
265	9					
139	8					
139	10					
139	11					
719	8					
61	9					
61	11					
695	11					
971	9	0.0001				
971	10	0.0001				
238	8					
395	8					
395	9					
645	8					
645	10					
645	11					
1123	9					
1123	10					
717	9					
717	10					
693	8					
693	9					
874	11					
40	10					
401	10					

Table VIII
HER2/NEU A02 Supermotif with Binding Data

Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
79	8					
79	9					
352	8					
352	10					
321	8					
364	11					
376	11					
73	8					
534	8					
425	11					
476	10	0.0001				
986	9	0.0002				
1093	9	0.0001				
1093	11					
1202	11					
19	9					
19	11					
621	8					
621	11					
729	10	0.0001				
1080	11					
919	8					
919	10					
704	9	0.0002				
704	11					
1231	10					
131	10	0.0001				
1164	9					
1164	10	0.0002				
1189	9	0.0002				
1189	10					
439	10	0.0030				
262	9					
262	10	0.0005				
262	11					
787	8					
787	10	0.0004				
787	11					
672	11					
660	8					
660	10	0.0007				
660	11					
925	10	0.0001				
925	11					
449	10	0.0003				
737	10	0.0002				
508	9	0.0120	0.0001	0.0790	0.0001	0.0044
464	9					
464	11					
865	8					

Table VIII

HER2/NEU A02 Supermotif with Binding Data

Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
865	11					
1062	9					
447	9	0.0018				
344	10	0.0017				
10	11					
549	9					
136	10	0.0001				
136	11					
454	8					
346	8					
346	10					
346	11					
1091	8					
1091	11					
832	8					
832	10	0.0017				
832	11					
537	10					
537	11					
28	8					
28	10					
1239	11					
104	10					
104	11					
732	9					
776	10	0.0001				
776	11					
603	9					
603	11					
152	10	0.0036				
909	8					
909	9					
668	8					
1179	8					
878	9	0.0002				
473	8					
42	8					
349	8					
349	9					
349	11					
48	8					
48	9	0.0340				
490	8					
901	10					
901	11					
495	11					
478	8					
858	9	0.0002				
858	10					
809	9	0.0002				

Table VIII
HER2/NEU A02 Supermotif with Binding Data

Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
86	9					
86	11					
829	8					
829	11					
654	8					
654	9	0.0005				
654	10	0.0120				
654	11					
767	8					
767	9	0.0210	0.0001	0.0024	0.0012	0.0003
767	11					
435	9	0.2100				
435	10					
435	11					
673	10					
714	10	0.0001				
148	8					
661	9	0.0020				
661	10	0.0006				
954	8					
361	10					
77	8					
77	9					
77	10					
77	11					
989	9					
861	9					
861	10					
406	9					
762	10					
369	9	0.1500				
747	8					
747	9	0.0002				
681	10	0.0001				
681	11					
860	8					
860	10	0.0020				
860	11					
32	10					
1171	10					
1171	11					
722	9					
724	10					
724	11					
753	11					
883	8					
883	9	0.0002				
3	9					
3	10	0.0022				
3	11					

Table VIII
HER2/NEU A02 Supermotif with Binding Data

Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
846	8					
509	8					
253	11					
465	8					
465	10					
13	8					
13	9					
13	11					
179	8					
1075	11					
866	10					
114	10	0.0002				
85	10	0.0001				
183	9					
467	8					
467	11					
674	9					
154	8					
12	9	0.0008				
12	10	0.0006				
869	11					
1008	10	0.0001				
1008	11					
934	8					
785	9					
785	10	0.0490				
11	10	0.0054				
11	11					
822	8					
822	9	0.0046				
15	9	0.0007				
15	11					
690	8					
690	11					
662	8					
662	9	0.0001				
800	8					
800	11					
74	11					
691	10					
691	11					
445	9					
445	11					
547	9					
547	11					
140	9					
140	10					
392	8					
392	11					
96	10					

Table VIII
HER2/NEU A02 Supermotif with Binding Data

Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
1109	9					
1109	10					
397	10					
397	11					
428	8					
428	9					
1131	10					
145	9					
145	10					
145	11					
181	11					
443	8					
443	11					
1197	8					
700	11					
215	11					
651	8					
651	9					
651	11					
790	8					
790	9					
790	11					
62	8					
62	10					
313	10	0.0002				
313	11					
1017	8					
1017	9	0.0030				
696	10					
696	11					
841	8					
841	11					
852	8					
852	10					
852	11					
972	8	0.0001				
972	9	0.0001				
796	11					
271	8					
663	8					
663	11					
774	9	0.0014				
979	9	0.0001				
979	10					
979	11					
953	8					
953	9	0.0051				
45	11					
827	10					
916	11					

Table VIII
HER2/NEU A02 Supermotif with Binding Data

Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
1042	11					
68	10	0.0001				
360	11					
59	9					
59	11					
427	9					
427	10	0.0001				
708	8					
708	11					
319	10					
177	8					
177	10					
89	8					
89	11					
388	10					
275	8					
471	9					
471	10					
758	10					
758	11					
125	8					
745	8					
745	10	0.0001				
745	11					
850	10	0.0001				
1158	8					
1158	9					
1158	10	0.0001				
643	9	0.0001				
643	10					
1211	10	0.0001				
1162	11					
269	8					
269	10					
1035	8					
1035	9					
927	8					
927	9	0.0001				
385	8					
36	8					
36	10	0.0001				
36	11					
996	8					
945	9					
945	10					
945	11					
885	10					
885	11					
627	9	0.0002				
627	11					

Table VIII
HER2/NEU A02 Supermotif with Binding Data

Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
612	11					
1074	8					
999	10	0.0001				
999	11					
316	8					
316	9					
122	8					
122	9					
122	11					
1156	8					
1156	10					
1156	11					
1119	10	0.0001				
1119	11					
391	9	0.0002				
95	8					
95	11					
1108	10					
1108	11					
1130	11					
699	8					
650	8					
650	9	0.0015				
650	10	0.0003				
159	8					
159	9					
159	10					
1135	11					
1205	8					
942	8					
942	10					
1147	11					
1026	11					
1241	9					
1241	11					
232	10					
232	11					
1102	8					
66	9					
525	11					
1116	9					
749	11					
128	10	0.0001				
709	10					
828	9	0.0006				
178	9					
160	8					
160	9	0.0001				
106	8					
106	9	0.4600				

Table VIII
HER2/NEU A02 Supermotif with Binding Data

Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
106	10	0.0140	0.0065	1.1000	0.0170	0.5400
106	11					
484	9	0.0062				
484	10	0.0003				
484	11					
799	8					
799	9	0.0230	0.0044	0.0880	0.0052	0.0031
396	8					
396	11					
141	8					
141	9	0.0008				
711	8					
711	10	0.0001				
1027	10					
679	8					
679	9					
24	8					
24	10	0.0001				
398	9					
398	10					
429	8					
93	9					
93	10	0.0001				
90	10	0.0001				
54	9	0.0001				
54	10					
190	9	0.0001				
647	8					
647	9	0.0002				
647	11					
354	8					
434	10	0.0180				
434	11					
713	8					
713	11					
100	8					
816	8					
868	8					
784	8					
784	10					
784	11					
689	8					
689	9	0.0910				
34	8					
34	10	0.0001				
940	9	0.0002				
940	10					
98	8					
98	10	0.0001				
840	8					

Table VIII
HER2/NEU A02 Supermotif with Binding Data

Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
840	9	0.0001				
978	10	0.0020				
978	11					
678	9					
678	10					
92	8					
92	10					
92	11					
217	9					
340	8					
340	11					
545	11					
358	8					
656	8					
656	9					
656	10	0.0009				
656	11					
413	10	0.0002				
653	9	0.0720	0.0082	0.2900	0.0130	0.2700
653	10	0.0002				
653	11					
893	8					
1007	8					
1007	11					
418	11					
1100	10	0.0059				
457	9	0.0002				
457	10					
457	11					
70	8					
70	11					
144	10	0.0150				
144	11					
442	9	0.0003				
214	8					
281	10					
819	9					
819	11					
532	10					
305	8					
305	9					
305	10	0.0001				
1235	8					
1235	9					
1002	8					
22	8					
22	10					
1051	9					
1051	10					
1051	11					

Table VIII
HER2/NEU A02 Supermotif with Binding Data

Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
792	9					
792	10					
423	8					
423	9	0.0017				
573	9					
297	8					
297	10					
1242	8					
1242	10	0.0001				
496	10					
389	9	0.0002				
389	11					
948	8					
948	9	0.0005				
402	9	0.0018				
402	11					
1166	8					
1060	11					
182	10					
444	10	0.0011				
1172	9	0.0002				
1172	10	0.0001				
312	11					
686	9					
686	11					
526	10					
105	9					
105	10					
105	11					
798	9					
798	10					
23	9					
23	11					
218	8					
1117	8					
793	8					
793	9					
911	10					
733	8					
750	10					
597	8					
988	10					
430	11					
116	8					
116	11					
725	9					
725	10	0.0007				
666	8					
666	9	0.0005				
666	10					

Table VIII
HER2/NEU A02 Supermotif with Binding Data

Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
84	8					
84	11					
153	9	0.0290				
546	10	0.0009				
754	10					
851	9	0.0002				
851	11					
773	10	0.0180				
56	8					
56	10					
80	8					
794	8					
296	9					
296	11					
574	8					
129	9					
797	10					
797	11					
356	10					
910	8					
910	11					
272	11					
658	8					
658	9	0.0005				
658	10	0.0009				
987	8					
987	11					
1180	11					
665	9	0.3500	0.0001	0.0040	0.0086	0.0200
665	10	0.0027				
665	11					
55	8					
55	9					
55	11					
664	10	0.0032				
664	11					
739	8					
739	10					
452	10	0.0001				
888	8					
411	9	0.0003				
835	8					
1248	8					
64	8					
64	11					
303	10	0.0002				
303	11					
1196	8					
1196	9	0.0001				
409	11					

Table VIII
HER2/NEU A02 Supermotif with Binding Data

Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
952	9	0.0230	0.0001	0.0160	0.0014	0.0400
952	10	0.0600	0.0004	0.0300	0.0190	0.0011
163	10					
50	11					
289	8					
289	9					
289	10					
685	10					
83	9	0.0005				
772	11					
554	8					
781	8					
781	10	0.0004				
781	11					

Table IX
HER2/NEU A03 Supermotif with Binding Data

Position	No. of Amino Acids	A*0301	A*1101	A*3101	A*3301	A*6801
241	8					
847	8					
1159	11					
890	8					
890	9	0.0013	0.0006			
492	8					
180	9	0.0004	0.0005			
180	11					
705	9	0.0004	0.0006			
37	11					
997	10	0.0003	0.0670	0.1200	0.0140	0.0520
240	9	0.0021	0.0021			
220	9	-0.0002	-0.0002			
805	10	0.0003	0.0001			
195	9	-0.0008	-0.0001			
26	9	0.0002	0.0005			
630	11					
947	11					
584	8					
596	10	0.0220	0.0042	0.0008	0.0064	0.0093
528	9	0.0015	0.0310	0.5300	0.5800	0.4400
845	10	0.0018	0.0007			
1089	8					
933	8					
821	11					
607	9	0.0005	0.0100	0.0002	0.0880	0.0310
962	9	-0.0002	-0.0002			
165	11					
1144	10	0.0003	0.0001			
950	8					
147	11					
930	8					
930	11					
914	8					
971	8					
971	11					
280	9	0.0003	-0.0002			
207	11					
717	8					
874	10	0.0003	0.0001			
40	8					
321	10	0.0002	0.0001			
976	10	-0.0002	0.0010			
729	8					
1038	9	-0.0002	0.0043			
1038	11					
919	11					
704	10	-0.0002	0.0041			
1231	8					
131	8					

Table IX
HER2/NEU A03 Supermotif with Binding Data

Position	No. of Amino Acids	A*0301	A*1101	A*3101	A*3301	A*6801
1164	8					
672	10	0.0150	0.0014			
449	8					
449	11					
737	11					
508	10	0.0110	0.0001			
1062	11					
447	10	0.0037	0.0001			
344	8					
344	11					
549	10	0.0002	0.0003			
136	8					
346	9	-0.0002	-0.0002			
832	9	-0.0002	0.0002			
1041	8					
727	10	0.0660	0.1300	0.0014	-0.0013	0.0012
327	10	0.0210	0.6100	0.0140	0.0012	0.0100
776	9	0.0010	0.0066			
668	9	0.0047	0.0890	0.0019	0.0025	0.0011
668	10	0.0180	0.0330	0.0590	0.0140	0.4300
668	11					
878	10	0.0003	0.0008			
632	9	-0.0002	0.0007			
478	10	0.0035	0.0720	0.9600	0.3300	2.0000
858	11					
809	8					
673	9	0.3800	0.0097	0.0760	0.0064	0.0001
673	11					
714	8					
714	9	0.0190	0.0023	0.0009	0.0010	0.0001
714	11					
148	10	0.0400	0.0005	0.7300	0.2400	0.0390
167	9	0.2800	0.3100	0.2200	0.0300	0.0046
450	10	0.0410	0.0027	2.6000	0.1300	0.1100
861	8					
747	10	0.0009	0.0099			
681	8	0.0010	0.0004			
681	9	0.7600	0.0018	1.1000	0.0072	0.0002
860	9	0.1700	0.2400	0.1800	0.0012	0.0049
753	10	0.3800	0.2200	0.0068	0.0012	0.0008
846	9	0.0580	0.0285	-0.0005	-0.0012	0.0160
509	9	-0.0002	0.0003			
179	10	-0.0002	0.0003			
85	8					
183	8					
674	8					
674	10	0.0002	0.0001			
674	11					
806	9	0.0370	0.0006	0.0360	0.0890	0.0014
806	11					

Table IX
HER2/NEU A03 Supermotif with Binding Data

Position	No. of Amino Acids	A*0301	A*1101	A*3101	A*3301	A*6801
822	10	0.1400	0.1400	0.0100	0.0088	0.0086
1173	10	-0.0002	0.0003			
422	11					
608	8					
181	8					
181	10	0.0002	0.0005			
841	9	0.0040	0.0014			
852	9	0.4800	0.0700	0.0990	0.0370	0.1100
972	10	0.0072	0.0330	0.3700	0.2300	0.2200
774	11					
889	8					
889	9	0.0034	0.0237	0.0940	0.2200	0.0630
889	10	0.0011	0.0003			
960	9	0.0017	0.0006			
960	11					
833	8					
360	9	0.0002	0.0036			
360	10	0.0003	0.0056			
427	8					
758	8					
745	9	0.0058	0.0007	0.0015	0.0820	0.1200
850	11					
1162	8					
1162	10	-0.0002	-0.0002			
927	11					
996	11					
999	8					
95	9	0.0002	0.0001			
1065	8					
1102	10	0.0003	0.0001			
749	8					
128	11					
491	9	0.0046	0.0010			
709	8					
178	11					
160	11					
141	10	0.2000	0.0130	0.0270	0.0047	0.0002
711	11					
24	9	0.0007	0.0520	0.0002	0.0006	0.0110
24	11					
93	8					
93	11					
90	9	0.0029	0.0005			
90	11					
190	11					
713	9	0.0007	0.0038			
713	10	0.0570	0.1100	0.0055	0.0013	0.0002
840	10	0.1800	0.0001	0.9500	0.0021	0.0036
978	8					
143	8					

Table IX
HER2/NEU A03 Supermotif with Binding Data

Position	No. of Amino Acids	A*0301	A*1101	A*3101	A*3301	A*6801
217	10	0.0068	0.0130	0.4500	0.0220	0.0250
545	8					
358	11					
281	8					
208	10	-0.0002	0.0020			
22	11					
423	10	0.0170	0.0750	0.0340	0.0390	0.2500
323	8					
323	11					
948	10	0.0130	0.1200	0.0018	0.0120	0.0250
166	10	0.0430	3.6000	0.0370	0.0420	0.0400
182	9	0.0004	0.0005			
1172	11					
218	9	0.0004	0.0230	0.1400	0.0890	0.0970
218	11					
479	9	0.0006	0.0072			
911	11					
597	9	0.0100	-0.0002			
666	11					
84	9	0.0033	0.0007			
754	9	0.4000	0.0130	0.1400	0.1000	0.0001
851	10	0.0820	0.0072	0.0052	0.0032	0.0005
973	9	-0.0002	0.0021			
322	9	0.0002	0.0140	0.0011	0.0037	0.1000
129	10	0.0002	0.0005			
669	8					
669	9	0.1100	0.7200	1.4000	0.3700	2.0000
669	10	0.0030	0.0160	0.0620	0.1500	0.5400
739	9	0.0002	0.0001			
452	8					
888	9	-0.0002	-0.0002			
888	10	0.0085	0.0016			
888	11					
959	8					
959	10	-0.0002	0.0002			
835	10	0.0003	0.0001			
83	10	0.0043	0.0013			
1139	8					

Table X

HER2/NEU A24 Supermotif Peptides with Binding Data

Position	No. of Amino Acids	A*2401
1216	8	0.0039
1186	11	
730	9	0.0002
730	10	0.0010
730	11	0.0008
1212	9	0.0011
1212	10	
1212	11	
113	11	
5	8	
5	9	
5	11	
890	10	
466	9	
466	11	
270	10	
705	8	
705	10	0.0002
705	11	-0.0003
1165	9	
1190	8	
115	9	
355	10	
657	11	
414	9	0.0041
440	9	0.1300
440	11	0.0230
771	11	
475	8	0.0190
475	11	0.0003
255	9	
789	8	
826	8	
826	10	
826	11	-0.0003
244	10	
26	8	
26	10	
630	8	
947	8	
947	9	
540	8	
540	11	
504	11	
528	8	
295	9	
295	10	
342	9	0.0180
162	8	0.0120
162	11	0.0016

Table X

HER2/NEU A24 Supermotif Peptides with Binding Data

Position	No. of Amino Acids	A*2401
863	8	0.0005
863	10	0.0002
171	9	
171	11	
76	8	
76	10	
76	11	
845	8	
1089	10	
993	8	
933	9	
821	9	
421	8	
421	11	
607	8	
607	10	
1016	8	
1016	9	
1013	11	
165	8	
165	9	
1084	9	
307	11	
838	9	
904	10	
950	10	
950	11	
363	8	-0.0003
363	9	0.0003
766	9	
147	8	
147	9	
405	8	
405	11	
2	8	
2	10	
2	11	
460	8	
460	9	
265	10	
914	10	
139	10	
139	11	
719	8	
61	11	
971	9	
1123	9	
717	10	
693	8	
40	10	

Table X
HER2/NEU A24 Supermotif Peptides with Binding Data

Position	No. of Amino Acids	A*2401
40	11	
401	9	
401	10	
401	11	
79	8	
352	10	
876	11	-0.0003
1022	9	0.0014
1022	10	0.0120
553	9	0.0061
73	11	
899	8	
899	10	
476	10	
476	11	
986	9	
1004	11	
262	10	
787	10	
672	11	
660	8	
925	10	
925	11	
449	10	
464	11	
865	8	
447	9	
136	10	
454	8	
1091	8	-0.0003
1091	11	-0.0003
832	10	
28	8	
1239	10	
1239	11	
104	9	
104	11	
732	8	
732	9	
776	10	
776	11	
603	8	
603	9	
603	11	
152	10	
909	8	
909	10	
668	8	
1179	9	
408	8	0.0044

Table X
HER2/NEU A24 Supermotif Peptides with Binding Data

Position	No. of Amino Acids	A*2401
257	10	0.0002
473	10	
42	8	
42	9	
478	8	
478	9	
858	9	
809	9	
370	8	0.0120
172	8	-0.0003
172	10	0.0022
654	8	
654	9	
654	10	
767	8	
435	9	
435	11	
673	10	
148	8	
661	11	
954	8	0.0210
861	9	
861	10	
406	10	
101	8	
738	11	0.0027
369	8	
369	9	
747	9	
681	10	
681	11	
860	10	
860	11	
722	10	
724	8	
883	9	
887	8	0.0080
887	9	0.0150
684	8	0.0024
107	8	0.0006
107	11	0.0006
485	9	0.0002
485	10	0.0014
467	8	
467	10	
674	9	
154	8	
154	10	
869	9	
1008	10	

Table X
HER2/NEU A24 Supermotif Peptides with Binding Data

Position	No. of Amino Acids	A*2401
934	8	
822	8	
690	11	
662	10	
800	8	0.0076
915	9	0.0001
1131	9	
145	10	
145	11	
443	8	
443	10	
443	11	
651	11	
790	11	
62	10	
1017	8	
696	11	
852	10	
1024	8	
972	8	
796	8	
796	11	
271	9	
663	9	
663	11	
410	10	0.0840
960	10	
953	8	
953	9	
916	8	
916	11	
360	11	
427	9	
427	10	
388	10	
275	8	
758	10	
758	11	
745	8	
745	11	
824	10	0.0002
945	8	
945	9	
945	10	
945	11	
885	10	
885	11	
627	11	
999	10	
999	11	

Table X
HER2/NEU A24 Supermotif Peptides with Binding Data

Position	No. of Amino Acids	A*2401
1119	9	
391	10	
1130	10	
699	8	
197	9	0.0011
1241	8	
1241	9	
1241	11	
1102	8	
1102	11	
66	8	
66	9	
525	10	
525	11	
128	10	
922	10	0.0005
780	9	0.1700
780	11	0.0320
828	8	
828	9	
513	9	
160	8	
160	9	
160	10	
106	9	
484	10	
484	11	
799	8	
799	9	
396	8	
396	11	
141	8	
141	9	
795	9	
711	10	
24	8	
24	10	
398	9	
429	8	
93	9	
90	10	
54	9	
54	11	
968	9	0.0180
898	9	0.0110
898	11	
985	10	0.0002
434	8	
434	10	
713	8	

Table X
HER2/NEU A24 Supermotif Peptides with Binding Data

Position	No. of Amino Acids	A*2401
100	8	
100	9	
816	8	
816	10	
868	10	
689	8	
34	10	
940	10	
98	10	
98	11	
978	9	0.0032
340	8	
340	11	
545	10	
8	8	0.0250
8	9	1.3000
1111	10	0.0120
653	9	
653	10	
653	11	
373	9	
1007	8	
1007	11	
418	8	
418	11	
1100	10	
457	9	
457	11	
70	8	
144	11	
442	9	
442	11	
281	9	0.0001
281	11	0.0180
305	9	
1002	8	
22	10	
1051	9	
1051	11	
792	9	
792	10	
423	9	
451	8	-0.0003
451	11	0.0036
907	9	0.1200
907	10	0.0630
834	8	0.0059
609	8	0.3200
917	10	0.0002
948	8	

Table X
HER2/NEU A24 Supermotif Peptides with Binding Data

Position	No. of Amino Acids	A*2401
166	8	
402	8	
402	9	
402	10	
402	11	
1166	8	
444	9	
444	10	
686	11	-0.0003
1117	11	
479	8	
793	8	
793	9	
793	11	
911	8	
733	8	
63	9	0.0380
63	11	8.9000
273	10	0.0074
1085	8	
399	8	-0.0003
399	11	
424	8	-0.0003
116	8	
725	11	
666	8	
666	9	
666	10	
153	9	
153	11	
546	9	
851	11	
773	9	0.0001
296	8	
296	9	
129	9	
797	10	
797	11	
356	9	
910	9	
272	8	
272	11	
658	10	
987	8	
1180	8	
665	9	
665	10	
665	11	
55	8	
55	10	

Table X
HER2/NEU A24 Supermotif Peptides with Binding Data

Position	No. of Amino Acids	A*2401
55	11	
664	8	
664	10	
664	11	
905	9	0.0800
905	11	0.0920
951	9	0.1600
951	10	0.0220
951	11	1.8000
739	10	
452	10	
888	8	-0.0003
959	11	0.0011
411	9	
64	8	
64	10	
64	11	
303	11	
1023	8	
1023	9	
409	11	
952	8	0.0009
952	9	
952	10	0.0019
772	10	0.0001
554	8	
781	8	
781	10	

Table XI
HER2/NEU B07 Supermotif Peptides with Binding Data

Position	No. of Amino Acids	B*0702
1036	8	0.0063
390	8	-0.0006
390	10	0.0001
390	11	0.0011
1129	11	-0.0002
1204	9	0.0056
1204	10	0.0530
1076	10	0.0002
1076	11	0.0006
642	10	0.1500
1032	8	-0.0002
1032	11	-0.0002
626	10	0.0002
315	8	-0.0006
315	10	0.0001
600	9	0.0140
600	11	0.0300
299	10	0.0016
1034	9	0.0001
1034	10	0.0002
384	9	0.0004
121	9	0.0002
982	8	-0.0006
1105	8	-0.0006
698	8	-0.0002
698	9	0.0110
995	10	0.0510
995	11	0.0036
578	8	-0.0006
578	9	0.0001
578	11	-0.0003
522	8	-0.0006
246	8	0.0092
246	9	0.0001
246	11	0.0006
1155	9	0.0900
1155	11	0.0160
524	11	0.0005
564	11	-0.0002
1208	9	0.0093
1208	10	0.0018
926	9	0.0006
926	10	0.0004
740	9	0.0001
740	11	0.0023
931	11	-0.0002
748	8	0.0120
336	8	-0.0006
336	10	0.0370
605	9	0.0720

Table XI
HER2/NEU B07 Supermotif Peptides with Binding Data

Position	No. of Amino Acids	B*0702
605	10	0.0001
921	8	0.0150
921	11	0.0430
1157	9	0.0027
1157	11	0.0140
35	9	0.0002
35	11	-0.0002
419	10	0.0003
377	10	0.0001
16	10	0.0002
941	9	0.0280
941	11	0.0032
550	8	0.0012
1120	8	-0.0006
1120	9	0.0002
1120	10	0.0001
231	11	-0.0003
1101	9	0.0460
65	9	0.0260
65	10	0.0190
282	8	-0.0006
282	10	0.0001
706	9	0.0090
706	10	0.0490
801	10	0.0085
284	8	-0.0002
284	10	0.0001
1245	9	0.0001
1245	11	-0.0002
1193	11	-0.0003
488	10	0.0005
158	10	0.0001
158	11	-0.0002
1210	8	-0.0002
1210	11	-0.0002
1227	11	-0.0003
17	9	0.0001
944	8	-0.0006
944	9	0.0001
944	10	0.0004
944	11	0.0064
1209	8	-0.0002
1209	9	0.0002
1149	9	0.0054
1149	11	0.4500
1233	11	-0.0003
393	8	-0.0002
393	11	-0.0002
1136	10	0.0001
1206	8	0.0002

Table XI
HER2/NEU B07 Supermotif Peptides with Binding Data

Position	No. of Amino Acids	B*0702
1206	11	0.0003
943	9	0.0001
943	10	0.0001
943	11	0.0020
1148	10	0.0014
568	10	0.0004
499	11	-0.0002
966	8	0.0410
966	11	1.3000
1214	8	-0.0002
1214	9	0.0001
1214	10	0.0001
38	8	0.0014
38	9	0.0005
133	8	0.0550
133	10	0.0580
1174	8	0.0230
1174	11	-0.0002
760	8	0.0580
760	9	0.1200
1073	9	0.0030
998	8	-0.0006
998	11	0.0640
649	9	0.0150
649	10	0.0900
649	11	0.0250
196	10	0.0021
855	10	0.0016
1151	9	0.6400
1151	10	0.4600
779	8	0.0440
779	10	0.1000
701	10	0.0001
1240	9	
1240	10	0.0002
127	11	-0.0002
884	8	1.4000
884	11	0.0017
1118	10	0.0001
1118	11	-0.0002
94	8	0.0020
94	9	0.0077
415	8	0.0200
415	10	0.0044
415	11	0.0005

Table XII
HER2/NEU B27 Supermotif Peptides

Position	No. of Amino Acids
87	10
588	8
598	11
980	10
928	8
867	11
1160	8
339	9
511	11
367	8
367	10
367	11
965	8
544	11
7	9
7	10
808	10
936	11
1229	9
939	8
939	11
173	9
173	11
969	8
969	11
486	8
486	9
1176	10
882	8
882	10
433	9
433	11
815	8
815	9
815	11
469	8
174	8
174	10
174	11
843	10
468	9
675	8
675	11
886	9
886	10
431	11
682	9
682	10
248	9
248	11

Table XII
HER2/NEU B27 Supermotif Peptides

Position	No. of Amino Acids
368	9
368	10
676	10
266	9
256	8
256	11
436	8
436	10
458	8
458	10
458	11
137	9
99	9
99	10
33	11
455	11
142	8
712	9
687	10
259	8
857	8
857	10
764	9
764	11
813	10
813	11
1207	11
761	8
247	10
551	11
967	10
680	8
680	11
646	9
646	10
984	11
97	11
156	8
1110	11
721	11
683	8
683	9
897	10
688	9
677	9
677	11
896	11
335	9
335	11
189	10

Table XII

HER2/NEU B27 Supermotif Peptides

Position	No. of Amino Acids
783	8
977	10
1103	10
472	11
41	9
41	10
900	9
1052	8
1052	10
842	11
477	9
477	10
746	10
859	8
859	11
604	8
604	10
604	11
853	9
723	9
353	9
810	8
102	11
839	8
91	9
91	11
169	11
877	10
1005	10

Table XIII
HER2/NEU B58 Supermotif Peptides

Position	No. of Amino Acids
1094	8
4	8
4	9
4	10
1203	11
1159	9
293	9
293	11
69	9
37	9
37	10
132	9
132	11
997	8
997	9
648	8
648	11
21	11
1165	9
587	9
587	11
224	8
338	8
338	10
334	8
334	10
195	11
1133	8
531	11
244	10
26	8
26	10
630	8
947	8
947	9
947	10
962	8
962	11
417	8
417	9
1001	8
1001	9
165	8
165	9
580	11
770	8
892	8
280	10
207	9
1123	9

Table XIII
HER2/NEU B58 Supermotif Peptides

Position	No. of Amino Acids
717	9
717	10
693	8
874	11
40	10
40	11
401	9
401	10
401	11
364	8
364	11
1213	8
1213	9
1213	10
1213	11
976	11
899	8
899	10
1093	9
621	8
729	10
729	11
1080	11
919	8
704	9
704	11
292	10
131	10
1164	10
1189	8
1189	9
439	10
309	9
1082	9
1082	11
727	8
727	9
372	10
778	8
778	9
778	11
818	8
818	10
28	8
1239	10
1239	11
104	9
104	11
732	8
732	9

Table XIII
HER2/NEU B58 Supermotif Peptides

Position	No. of Amino Acids
878	9
878	11
249	8
249	10
495	11
478	8
478	9
86	9
86	11
829	8
829	11
655	8
655	9
655	10
655	11
412	8
412	11
450	9
861	9
861	10
406	10
762	11
854	8
854	11
1171	10
1171	11
3	9
3	10
3	11
846	8
253	11
374	8
465	10
1075	11
114	10
71	10
1173	8
1173	9
304	10
304	11
422	9
422	10
608	9
1131	9
1131	10
145	9
145	10
145	11
443	8
443	10

Table XIII
HER2/NEU B58 Supermotif Peptides

Position	No. of Amino Acids
443	11
651	8
651	9
651	11
790	8
790	11
62	10
774	8
774	9
889	11
979	8
979	9
979	10
979	11
833	9
833	10
916	8
916	11
68	10
388	10
275	8
471	10
758	10
758	11
1158	10
643	9
1215	8
1215	9
1211	10
1211	11
269	11
1035	8
1035	9
927	8
927	9
385	8
36	8
36	10
36	11
996	9
996	10
1065	11
1077	9
1077	10
1121	8
1121	11
702	11
601	8
601	10
601	11

Table XIII
HER2/NEU B58 Supermotif Peptides

Position	No. of Amino Acids
1150	8
1241	8
1241	9
1241	11
1102	8
1102	11
66	8
66	9
525	10
525	11
902	9
902	11
1099	11
190	9
647	8
647	9
354	8
354	11
1053	9
1053	11
1006	9
456	10
143	11
188	11
656	8
656	9
656	10
656	11
413	10
208	8
1049	11
1050	10
305	9
305	10
1002	8
22	10
1051	9
1051	11
792	9
792	10
297	8
1242	8
1242	10
496	10
389	9
389	11
357	8
652	8
652	10
652	11

Table XIII
HER2/NEU B58 Supermotif Peptides

Position	No. of Amino Acids
759	9
759	10
791	10
791	11
585	11
597	8
782	9
296	8
296	9
129	9
797	10
797	11
356	9
910	9
272	8
272	11
906	8
906	10
906	11
1112	9
441	8
441	10
289	8

Table XIV
HER2/NEU B62 Supermotif Peptides

Position	No. of Amino Acids
890	10
466	11
270	10
705	8
705	10
1036	8
390	10
390	11
1129	11
1204	10
1076	10
1076	11
355	10
657	8
657	9
657	10
255	9
789	9
826	8
826	10
1032	11
626	10
315	8
600	11
299	10
567	8
567	11
212	8
596	9
295	9
76	8
76	9
76	11
845	9
821	9
421	10
421	11
607	8
607	10
1016	8
1016	10
1013	11
1034	9
1034	10
121	9
982	8
1105	8
582	9
1183	9
1084	9

Table XIV
HER2/NEU B62 Supermotif Peptides

Position	No. of Amino Acids
307	8
904	9
904	10
950	10
950	11
766	8
766	9
147	9
405	8
405	11
2	8
460	9
265	8
265	10
914	10
139	10
971	9
698	9
645	10
645	11
79	8
352	10
73	8
73	11
534	8
425	11
476	11
262	11
787	8
787	11
672	11
660	10
660	11
737	10
865	8
344	10
346	8
346	11
832	8
832	11
995	10
995	11
578	8
522	8
524	11
537	10
603	8
603	9
603	11
909	8

Table XIV
HER2/NEU B62 Supermotif Peptides

Position	No. of Amino Acids
909	10
668	8
1179	9
473	8
473	10
42	9
349	8
48	8
48	9
564	11
1208	10
512	10
901	8
901	10
654	8
654	11
767	8
767	11
673	10
714	10
148	8
661	9
661	10
661	11
954	8
740	9
740	11
361	10
361	11
77	8
77	10
155	9
101	8
369	8
747	8
336	8
605	9
605	10
921	11
722	10
724	8
724	11
85	10
467	10
467	11
674	9
154	10
869	9
1008	11
785	10

Table XIV
HER2/NEU B62 Supermotif Peptides

Position	No. of Amino Acids
822	8
15	11
690	8
662	8
662	9
662	10
800	11
915	9
35	11
16	10
941	9
941	11
1120	8
1120	9
65	9
74	10
74	11
445	8
547	8
547	9
140	9
392	8
392	9
1109	10
397	10
428	8
313	10
1017	9
696	11
841	11
852	8
852	10
1024	8
972	8
796	8
271	9
663	8
663	9
663	11
960	10
953	8
953	9
45	11
282	8
282	10
706	9
801	10
827	9
360	11
427	9

Table XIV
HER2/NEU B62 Supermotif Peptides

Position	No. of Amino Acids
284	8
1245	9
1245	11
158	10
177	8
745	8
745	10
850	10
945	8
945	9
945	10
945	11
885	10
627	9
122	8
1119	9
1119	10
391	9
391	10
95	8
1108	11
1130	10
1130	11
699	8
650	9
650	10
197	9
1210	8
1227	11
17	9
944	8
944	9
944	10
944	11
1209	9
159	9
159	11
569	9
1135	11
1205	9
942	8
942	10
942	11
828	8
513	9
160	8
160	10
106	11
396	11
141	8

Table XIV
HER2/NEU B62 Supermotif Peptides

Position	No. of Amino Acids
795	9
393	8
1136	10
1206	8
943	9
943	10
943	11
568	10
679	9
24	8
398	9
93	9
93	10
54	11
434	8
713	11
100	9
816	10
868	10
784	11
689	9
940	10
98	11
978	9
978	10
978	11
966	8
966	11
678	8
678	10
92	10
92	11
340	8
545	10
545	11
653	9
373	9
1007	8
418	8
70	8
70	11
144	10
442	9
442	11
281	9
281	11
1214	8
1214	9
1214	10
38	8

Table XIV

HER2/NEU B62 Supermotif Peptides

Position	No. of Amino Acids
1174	8
1174	11
760	8
998	8
649	10
649	11
196	10
855	10
779	10
819	9
819	11
532	10
423	8
423	9
948	8
948	9
166	8
402	8
402	10
402	11
444	9
1172	9
1172	10
312	11
1240	9
526	9
105	8
23	9
1117	11
479	8
793	9
793	11
911	8
733	8
725	10
725	11
666	8
666	10
84	8
84	11
153	11
546	9
546	10
851	9
851	11
773	9
773	10
884	11
1118	10
1118	11

Table XV
HER2/NEU B62 Supermotif Peptides

Position	No. of Amino Acids
94	8
94	9
56	9
794	8
794	10
658	8
658	9
1180	8
665	9
665	11
55	10
664	8
664	10
739	8
739	10
959	11
415	10
415	11
835	8
1248	8
64	10
1023	8
1023	9
952	8
952	9
952	10
163	10
163	11
83	9
772	10
772	11
781	8

Table XV
HER2/NEU A01 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*0101
1212	10	0.0010
1212	11	0.0140
293	9	0.0550
293	11	0.1900
997	9	0.0290
826	10	0.3000
600	11	
334	10	0.0016
1013	11	0.0027
1105	8	
580	11	0.1000
280	10	0.1800
40	11	0.2800
401	9	0.0430
401	11	0.4400
279	11	0.0049
291	11	0.0100
1213	9	0.0430
1213	10	5.5000
899	10	2.7000
292	10	0.0012
1188	9	
995	11	
727	9	0.0011
1239	10	0.0630
104	9	0.1800
42	9	9.1000
901	8	-0.0021
333	11	-0.0017
403	9	0.0057
726	10	0.0010
869	9	7.6000
915	9	0.0011
1120	8	
74	10	0.0015
1131	9	0.1300
1014	10	0.0120
916	8	-0.0021
764	9	0.0017
996	10	0.0150
601	10	0.0010
1241	8	0.0030
1102	11	0.0160
828	8	-0.0021
728	8	-0.0021
281	9	0.0028
1214	8	
1214	9	
1132	8	-0.0021
1103	10	0.0015

Table XV

HER2/NEU A01 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*0101
402	8	-0.0021
402	10	1.1000
399	11	0.0045
773	9	0.0400
296	8	0.1000

Table XVI
HER2/NEU A03 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*0301
241	8	
241	9	
1094	11	
4	11	
1203	10	
1203	11	
847	8	
1159	11	
586	10	
191	10	
510	8	
510	10	
622	11	
879	9	
581	8	
581	9	
581	10	
581	11	
1186	11	
1212	10	0.0003
1212	11	
1163	9	
365	11	
242	8	
221	8	
1039	8	
1039	9	
1039	10	
775	10	
5	10	
890	8	
890	9	0.0013
890	10	
466	8	
466	11	
492	8	
14	8	
180	9	0.0004
180	11	
270	10	
705	9	0.0004
293	9	0.0008
293	11	
37	11	
997	8	
997	9	0.0002
997	10	0.0003
648	10	
355	10	
355	11	

Table XVI

HER2/NEU A03 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*0301
240	9	0.0021
240	10	
220	9	-0.0002
587	9	
235	8	
576	11	
252	9	
805	10	0.0003
826	10	0.0003
334	10	0.0003
195	9	-0.0008
244	11	
26	9	0.0002
630	11	
947	11	
311	8	
584	8	
596	10	0.0220
634	11	
504	9	
528	9	0.0015
295	9	0.0002
234	8	
234	9	
251	8	
251	10	
211	11	
1011	10	
638	10	
638	11	
1012	9	
1087	8	
1087	9	
1087	10	
382	9	
742	10	
880	8	
880	11	
326	8	
326	11	
871	8	
871	9	
171	10	
76	8	
845	10	0.0018
636	9	
1089	8	
933	8	
821	10	
821	11	

Table XVI
HER2/NEU A03 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*0301
607	9	0.0005
607	10	
1016	8	
1013	8	
1013	11	
30	8	
962	8	
962	9	-0.0002
417	9	
165	9	
165	10	
165	11	
185	9	
1183	8	
1084	11	
838	10	
838	11	
1144	10	0.0003
950	8	
580	9	
580	10	
580	11	
1069	8	
543	10	
503	8	
503	10	
210	8	
1010	11	
501	10	
325	9	
837	8	
837	11	
363	9	
975	11	
1079	8	
507	10	
507	11	
1154	8	
1154	10	
286	8	
766	10	
147	11	
930	8	
930	11	
405	10	
460	10	
460	11	
265	10	0.0002
914	8	
914	10	0.0002

Table XVI
HER2/NEU A03 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*0301
61	9	
695	11	
971	8	
971	10	
971	11	
379	8	
892	8	
892	10	
280	9	0.0003
280	10	0.0003
207	11	
717	8	
874	10	0.0003
40	8	
40	9	
40	11	
401	9	0.0002
401	11	
79	9	
79	10	
352	8	
321	10	0.0002
364	8	
1031	9	
595	11	
1086	9	
1086	10	
1086	11	
381	10	
1030	10	
918	11	
291	8	
291	11	
1187	10	
671	8	
671	11	
577	10	
371	11	
376	11	
73	11	
1213	9	0.0002
1213	10	0.0005
976	10	-0.0002
976	11	
899	10	0.0003
476	11	
1202	11	
729	8	
1038	8	
1038	9	-0.0002

Table XVI
HER2/NEU A03 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*0301
1038	10	
1038	11	
919	10	
919	11	
704	10	-0.0002
1231	8	
292	10	0.0003
131	8	
1164	8	
1189	8	
366	10	
366	11	
804	8	
804	11	
641	8	
1088	8	
1088	9	
1015	9	
383	8	
1029	8	
1029	11	
1201	8	
1188	9	0.0003
881	10	
135	9	
1040	8	
1040	9	
262	9	
672	10	0.0150
449	8	
449	11	
737	11	
508	9	
508	10	0.0110
464	10	
1062	9	
1062	11	
447	10	0.0037
344	8	
344	11	
10	11	
549	9	
549	10	0.0002
549	11	
1097	8	
136	8	
346	9	-0.0002
346	10	
832	9	-0.0002
1041	8	

Table XVI

HER2/NEU A03 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*0301
369	10	
727	9	0.0028
727	10	0.0660
462	8	
462	9	
372	10	
572	10	
28	10	
1239	10	0.0002
104	9	0.0001
104	10	
327	10	0.0210
776	9	0.0010
603	8	
909	10	
668	9	0.0047
668	10	0.0180
668	11	
1179	8	
1179	9	
878	10	0.0003
267	8	
1104	8	
1104	9	
257	11	
42	9	0.0370
349	11	
632	9	-0.0002
249	9	
249	10	
260	8	
260	11	
478	9	
478	10	0.0035
858	10	
858	11	
809	8	
263	8	
872	8	
961	8	
961	9	
961	10	
184	8	
184	10	
949	9	
172	9	
767	9	
673	9	0.3800
673	11	
714	8	

Table XVI
HER2/NEU A03 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*0301
714	9	0.0190
714	11	
148	10	0.0400
661	11	
894	8	
167	8	
167	9	0.2800
450	10	0.0410
861	8	
406	9	
101	8	
762	10	
762	11	
333	8	
333	11	
957	10	
170	11	
591	8	
591	9	
1182	9	
615	8	
640	8	
640	9	
150	8	
1096	9	
831	10	
228	10	
1238	11	
369	8	
747	10	0.0009
681	8	0.0010
681	9	0.7600
860	8	
860	9	0.1700
32	11	
854	11	
722	9	
722	10	
724	8	
753	10	0.3800
753	11	
883	8	
846	9	0.0580
509	8	
509	9	-0.0002
509	11	
253	8	
374	8	
465	9	
13	8	

Table XVI
HER2/NEU A03 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*0301
13	9	
179	10	-0.0002
6	9	
161	10	0.0081
637	8	
637	11	
768	8	
807	8	
807	10	
870	8	
870	9	
870	10	
43	8	
107	9	
485	8	
485	11	
448	9	
1061	10	
345	10	
345	11	
994	11	
726	10	0.0003
726	11	
461	9	
461	10	
667	10	
667	11	
85	8	
183	8	
183	9	
183	11	
467	10	
674	8	
674	10	0.0002
674	11	
154	10	0.0012
12	9	
12	10	
806	9	0.0370
806	11	
869	9	0.0003
869	10	
869	11	
11	10	
11	11	
822	9	
822	10	0.1400
662	10	
800	10	
915	9	0.0002

Table XVI
HER2/NEU A03 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*0301
1173	10	-0.0002
422	11	
608	8	
608	9	
1131	9	0.0001
181	8	
181	10	0.0002
181	11	
1197	8	
700	11	
215	11	
62	8	
696	10	
841	8	
841	9	0.0040
852	9	0.4800
1024	8	
972	9	
972	10	0.0072
796	8	
271	9	
663	9	
774	8	
774	11	
889	8	
889	9	0.0034
889	10	0.0011
889	11	
979	8	
1014	10	0.0002
960	9	0.0017
960	10	
960	11	
833	8	
833	11	
916	8	
556	9	
571	11	
1178	8	
1178	9	
1178	10	
360	9	0.0002
360	10	0.0003
59	11	
427	8	
427	11	
471	8	
758	8	
125	8	
745	9	0.0058

Table XVI
HER2/NEU A03 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*0301
850	9	
850	11	
1158	8	
1215	8	
1211	11	
1162	8	
1162	10	-0.0002
269	11	
1035	10	
1035	11	
927	11	
996	9	
996	10	0.0003
996	11	
625	8	
194	10	
741	11	
932	9	
606	10	
606	11	
416	10	
1143	11	
1037	8	
1037	9	
1037	10	
1037	11	
134	10	
1175	8	
1175	11	
945	8	
612	11	
1074	8	
999	8	
316	9	
122	11	
1156	8	
1156	10	
1119	9	0.0002
1119	11	
230	8	
391	10	
95	9	0.0002
1130	10	0.0002
650	8	
1065	8	
1077	10	
1121	9	
702	9	
601	10	0.0003
1150	10	

Table XVI
HER2/NEU A03 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*0301
1150	11	
1234	10	
1241	8	
232	10	
232	11	
1102	10	0.0003
1102	11	
66	8	
525	10	
749	8	
128	11	
491	9	0.0046
709	8	
239	10	
239	11	
583	8	
583	9	
527	8	
527	10	
75	9	
820	11	
1225	8	
164	10	
164	11	
1028	9	
1200	9	
446	11	
548	10	
548	11	
57	8	
81	8	
828	8	
178	11	
160	11	
106	8	
106	10	
484	9	
799	11	
141	10	0.2000
795	9	0.0110
711	11	
213	9	
24	9	0.0007
24	11	
429	9	
93	8	
93	11	
90	9	0.0029
90	11	
54	11	

Table XVI
HER2/NEU A03 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*0301
190	11	
647	11	
354	11	
330	10	
330	11	
844	11	
968	9	
968	11	
898	11	
1230	8	
1230	9	
536	10	
432	9	
432	10	
103	10	0.0003
103	11	
434	8	
713	9	0.0007
713	10	0.0570
100	9	
868	10	0.0017
868	11	
34	9	
98	11	
840	8	
840	9	
840	10	0.1800
978	8	
978	9	0.0001
456	11	
143	8	
1072	10	
217	9	
217	10	0.0068
340	10	
545	8	
545	10	0.0350
358	8	
358	11	
310	9	
633	8	
294	8	
294	10	
250	8	
250	9	
250	11	
380	11	
728	8	
728	9	
703	8	

Table XVI
HER2/NEU A03 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*0301
703	11	
261	10	
463	8	
463	11	
602	9	
893	9	
373	9	
418	8	
457	10	
214	8	
281	8	
281	9	0.0002
281	11	
208	10	-0.0002
1235	9	
22	11	
423	10	0.0170
573	9	
323	8	
323	11	
1132	8	
233	9	
233	10	
29	9	
278	11	
290	9	
1236	8	
130	9	
245	10	
27	8	
27	11	
407	8	
948	10	0.0130
166	8	
166	9	
166	10	0.0430
402	8	
402	10	0.0001
1060	11	
182	9	0.0004
182	10	
1172	11	
357	8	
357	9	
218	8	
218	9	0.0004
218	11	
1117	11	
479	8	
479	9	0.0006

Table XVI
HER2/NEU A03 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*0301
793	11	
911	8	
911	10	
911	11	
585	11	
597	9	0.0100
219	8	
219	10	
314	11	
25	8	
25	10	
341	9	
341	11	
635	10	
1085	10	
1085	11	
399	11	
670	8	
670	9	
424	9	
424	11	
505	8	
308	11	
777	8	
988	10	
430	8	
430	11	
725	11	
666	11	
84	9	0.0033
153	11	
546	9	0.0012
754	9	0.4000
754	10	
851	8	
851	10	0.0820
773	9	0.0580
973	8	
973	9	-0.0002
296	8	
322	9	0.0002
574	8	
129	10	0.0002
356	9	
356	10	
910	9	
910	11	
272	8	
669	8	
669	9	0.1100

Table XVI

HER2/NEU A03 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*0301
669	10	0.0030
987	11	
1180	8	
1180	11	
55	10	0.0024
664	8	
825	11	
1223	8	
1223	10	
482	9	
482	11	
739	9	0.0002
452	8	
888	9	-0.0002
888	10	0.0085
888	11	
959	8	
959	10	-0.0002
959	11	
803	9	
341	9	
908	11	
835	9	
835	10	0.0003
64	10	
1196	8	
1196	9	
1023	8	
1023	9	
289	10	
83	10	0.0043
772	10	0.0100
554	11	
1139	8	

TableXVII
HER2/NEU A11 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*1101
241	8	
241	9	
1094	11	
847	8	
1159	11	
191	10	
510	8	
510	10	
622	11	
879	9	
581	9	
581	10	
581	11	
1186	11	
1212	10	0.0003
1212	11	
1163	9	
242	8	
221	8	
1039	8	
1039	9	
1039	10	
775	10	
890	8	
890	9	0.0006
466	8	
492	8	
180	9	0.0005
180	11	
705	9	0.0006
359	10	
359	11	
763	10	
293	9	0.0074
293	11	
37	11	
997	9	0.0004
997	10	0.0670
240	9	0.0021
240	10	
220	9	-0.0002
252	9	
805	10	0.0001
826	10	0.0001
334	10	0.0002
195	9	-0.0001
26	9	0.0005
630	11	
947	11	
311	8	

TableXVII
HER2/NEU A11 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*1101
584	8	
596	10	0.0042
504	9	
528	9	0.0310
295	9	0.0004
251	10	
638	10	
1087	10	
880	8	
326	8	
326	11	
871	8	
76	8	
845	10	0.0007
1089	8	
933	8	
821	11	
607	9	0.0100
1016	8	
1013	11	
962	9	-0.0002
165	10	
165	11	
185	9	
1144	10	0.0001
950	8	
580	10	
580	11	
543	10	
503	10	
210	8	
325	9	
837	8	
975	11	
507	11	
1154	8	
147	11	
930	8	
930	11	
460	10	
460	11	
265	10	0.0002
914	8	
914	10	0.0002
971	8	
971	11	
757	9	
744	10	
892	10	
280	9	-0.0002

TableXVII
HER2/NEU A11 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*1101
280	10	0.0003
207	11	
717	8	
874	10	0.0001
40	8	
40	9	
40	11	
401	9	0.0002
401	11	
79	10	
321	10	0.0001
595	11	
1086	11	
291	11	
1187	10	
671	8	
671	11	
73	11	
258	10	
1213	9	0.0002
1213	10	0.0010
976	10	0.0010
899	10	0.0005
729	8	
1038	8	
1038	9	0.0043
1038	10	
1038	11	
919	11	
704	10	0.0041
1231	8	
292	10	0.0001
131	8	
1164	8	
1189	8	
804	8	
804	11	
1088	9	
1015	9	
1201	8	
1188	9	0.0001
135	9	
1040	8	
1040	9	
672	10	0.0014
449	8	
449	11	
737	11	
508	10	0.0001
464	10	

TableXVII

HER2/NEU A11 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*1101
1062	11	
447	10	0.0001
344	8	
344	11	
549	10	0.0003
549	11	
1027	8	
136	8	
346	9	-0.0002
832	9	0.0002
1041	8	
309	10	
727	9	0.0001
727	10	0.1300
462	8	
462	9	
1239	10	0.0022
104	9	0.0280
327	10	0.6100
776	9	0.0066
603	8	
668	9	0.0890
668	10	0.0330
668	11	
878	10	0.0008
267	8	
1104	8	
1104	9	
257	11	
42	9	0.0002
88	11	
470	9	
632	9	0.0007
249	9	
260	8	
478	10	0.0720
858	11	
809	8	
961	8	
961	10	
184	10	
949	9	
673	9	0.0097
673	11	
714	8	
714	9	0.0023
714	11	
148	10	0.0005
894	8	
167	8	

TableXVII
HER2/NEU A11 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*1101
167	9	0.3100
450	10	0.0027
861	8	
762	11	
333	8	
333	11	
957	10	
591	9	
640	8	
150	8	
1096	9	
831	10	
228	10	
1238	11	
747	10	0.0099
681	8	0.0004
681	9	0.0018
860	9	0.2400
32	11	
753	10	0.2200
846	9	0.0285
509	9	0.0003
509	11	
253	8	
465	9	
179	10	0.0003
161	10	0.0063
637	11	
807	8	
807	10	
870	8	
870	9	
43	8	
485	11	
448	9	
345	10	
726	10	0.0003
726	11	
461	9	
461	10	
667	10	
667	11	
85	8	
183	8	
183	11	
674	8	
674	10	0.0001
674	11	
154	10	0.0002
806	9	0.0006

TableXVII
HER2/NEU A11 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*1101
806	11	
869	9	0.0001
869	10	
822	10	0.1400
800	10	
915	9	0.0003
823	9	
1173	10	0.0003
422	11	
608	8	
1131	9	0.0061
181	8	
181	10	0.0005
841	9	0.0014
852	9	0.0700
972	10	0.0330
796	8	
774	8	
774	11	
889	8	
889	9	0.0237
889	10	0.0003
1014	10	0.0002
960	9	0.0006
960	11	
833	8	
833	11	
916	8	
556	9	
360	9	0.0036
360	10	0.0056
427	8	
427	11	
471	8	
758	8	
745	9	0.0007
850	9	
850	11	
1215	8	
1211	11	
1162	8	
1162	10	-0.0002
1035	10	
1035	11	
927	11	
996	10	0.0001
996	11	
625	8	
194	10	
932	9	

TableXVII
HER2/NEU A11 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*1101
606	10	
1143	11	
1037	8	
1037	9	
1037	10	
1037	11	
134	10	
1175	8	
945	8	
999	8	
1119	9	0.0002
230	8	
95	9	0.0001
1130	10	0.0002
707	10	
1065	8	
601	10	0.0003
1241	8	
1102	10	0.0001
1102	11	
749	8	
128	11	
491	9	0.0010
709	8	
239	10	
239	11	
583	8	
583	9	
527	10	
75	9	
164	11	
1200	9	
446	11	
548	11	
57	8	
81	8	
828	8	
178	11	
160	11	
799	11	
141	10	0.0130
795	9	0.0039
711	11	
426	9	
24	9	0.0520
24	11	
429	9	
93	8	
93	11	
90	9	0.0005

TableXVII
HER2/NEU A11 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*1101
90	11	
54	11	
190	11	
330	11	
844	11	
968	11	
898	11	
1230	9	
536	10	
432	10	
103	10	0.0015
434	8	
713	9	0.0038
713	10	0.1100
868	10	0.0001
868	11	
34	9	
840	10	0.0001
978	8	
487	9	
849	10	
143	8	
217	10	0.0130
340	10	
545	8	
545	10	0.0050
358	11	
310	9	
633	8	
294	8	
294	10	
250	8	
250	11	
728	8	
728	9	
703	11	
463	8	
463	11	
602	9	
893	9	
281	8	
281	9	0.0003
208	10	0.0020
22	11	
423	10	0.0750
323	8	
323	11	
1132	8	
278	11	
130	9	

TableXVII
HER2/NEU A11 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*1101
27	8	
948	10	0.1200
166	9	
166	10	3.6000
402	8	
402	10	0.0001
182	9	0.0005
1172	11	
186	8	
218	9	0.0230
218	11	
1117	11	
479	9	0.0072
793	11	
911	11	
597	9	-0.0002
219	8	
219	10	
25	8	
25	10	
341	9	
341	11	
399	11	
670	8	
670	9	
424	9	
424	11	
505	8	
308	11	
777	8	
430	8	
725	11	
666	11	
84	9	0.0007
153	11	
546	9	0.0002
754	9	0.0130
851	8	
851	10	0.0072
773	9	0.0079
555	10	
529	8	
973	9	0.0021
296	8	
322	9	0.0140
129	10	0.0005
669	8	
669	9	0.7200
669	10	0.0160
55	10	0.0110

TableXVII
HER2/NEU A11 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*1101
825	11	
1223	8	
482	9	
739	9	0.0001
452	8	
888	9	-0.0002
888	10	0.0016
888	11	
959	8	
959	10	0.0002
803	9	
343	9	
835	9	
835	10	0.0001
83	10	0.0013
772	10	0.0120
554	11	
1139	8	

TableXVIII

HER2/NEU A24 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*2401
1216	8	0.0039
730	9	0.0002
730	10	0.0010
730	11	0.0008
1212	9	0.0011
705	10	0.0002
705	11	-0.0003
414	9	0.0041
440	9	0.1300
440	11	0.0230
475	8	0.0190
475	11	0.0003
826	11	-0.0003
342	9	0.0180
162	8	0.0120
162	11	0.0016
863	8	0.0005
863	10	0.0002
363	8	-0.0003
363	9	0.0003
876	11	-0.0003
1022	9	0.0014
1022	10	0.0120
553	9	0.0061
1091	8	-0.0003
1091	11	-0.0003
832	10	
408	8	0.0044
257	10	0.0002
370	8	0.0120
172	8	-0.0003
172	10	0.0022
954	8	0.0210
738	11	0.0027
887	8	0.0080
887	9	0.0150
684	8	0.0024
107	8	0.0006
107	11	0.0006
485	9	0.0002
485	10	0.0014
800	8	0.0076
410	10	0.0840
197	9	0.0011
922	10	0.0005
780	9	0.1700
780	11	0.0320
711	10	
968	9	0.0180
898	9	0.0110

TableXVIII
HER2/NEU A24 Motif Peptides with Binding Data

Position	No. of Amino Acids	A*2401
985	10	0.0002
978	9	0.0032
8	8	0.0250
8	9	1.3000
1111	10	0.0120
281	11	0.0180
451	8	-0.0003
451	11	0.0036
907	9	0.1200
834	8	0.0059
609	8	0.3200
917	10	0.0002
686	11	-0.0003
63	9	0.0380
63	11	8.9000
399	8	-0.0003
424	8	-0.0003
905	9	0.0800
905	11	0.0920
951	9	0.1600
951	11	1.8000
888	8	-0.0003
959	11	0.0011
952	8	0.0009
952	10	0.0019

Table XIX

HER2/NEU DR Super Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	Position	DR1	DR2w81	DR2w2B2	DR3	DR4w4	DR4w15	DR5w11	DR5w12	SEQ ID NO.
YNYLSTDVG	ACPYNYLSTDVGSCT	298									3720
VLRENTSPK	AIKVLRENTSPKANK	751				0.0075					3721
LQSLPTHDP	AKGLQSLPTHDPSPL	1095									3722
YDGIPAEI	AKPYDGIPAEHPDL	920									3723
LDIDETEYH	ARLLDIDETEYHADG	867	0.0001	-0.0006	-0.0007	0.3100	-0.0055		-0.0008		3724
VLVKSPNHV	ARNVLVKSPNHVKIT	848									3725
LTSHSAVV	ASPLTSHSAVVGIL	648	0.0890	0.0950	0.0037	0.0010	-0.0025		-0.0005		3726
LTLQGLGIS	AYSLTLQGLGISWLG	440									3727
MAGVGSPYV	AYVMAGVGSPYVSRL	771									3728
IFGSLAFLP	CKKIFGSLAFLPESF	367	0.2400	0.0070	0.0016	0.0010	-0.0025		-0.0005		3729
FNHSGICEL	CLHFNHSGICELHCP	255									3730
IAKGMSYLE	CMQIAKGMSYLEDVR	826									3731
LVTYNTDTF	CPALVTYNTDTFESM	268									3732
LTRTVCAGG	CQSLTRTVCAGGCAR	212									3733
LDDKGCPAE	CVDLDDKGCPAEQRA	634									3734
LGMEHLREV	CYGLGMEHLREVRAV	342				0.0083					3735
YVMAGVGSP	DEAYVMAGVGSPYVS	769	0.0500	0.0029	0.0240	0.0010	0.2300		0.0027		3736
VGEGLACHQ	DECVGEGLACHQLCA	502									3737
LGMGAAKGL	DGDLGMGAAKGLQSL	1087									3738
VAPLTCSPQ	DGYVAPLTCSPQPEY	1125									3739
LGLEPSEEE	DLTLGLEPSEEEAPR	1058				-0.0025					3740
LRLPASPET	DMKLRLPASPETHLD	30	0.0010				-0.0025				3741
FCVARCPSG	DPPFCVARCPSGVKP	592									3742
FYRSLLEDD	DSTFYRSLLEDDDMG	1001									3743
LVHRDLAAR	DVRLVHRDLAARNVL	838									3744
MIMVKCWMI	DVYMIMVKCWMIDSE	950	0.0280	0.0047	0.0042	0.0010	0.0570		0.0220		3745
VLQGLPREY	ECRVLQGLPREYVNA	543									3746
YALAVLDNG	EDNYALAVLDNGDPL	109									3747
LPAARPAGA	EGPLPAARPAGATLE	1154									3748
YTFGASCVT	EGRYTFGASCVTACP	286									3749
YLPTNASLS	ELTYLPTNASLSFLQ	61									3750
LRRRFTHQS	ESILRRRFTHQSDVW	892									3751
LRKVKVLGS	ETELRKVKVLGSGAF	717	0.0160	0.0019	0.0052	0.0045	0.0350		0.0061		3752
LVEPLTPSG	ETELVEPLTPSGAMP	693	0.0060				-0.0025				3753
LVSEFSRMA	FRELVSEFSRMARDP	969				0.0710					3754
IQNEDLGPA	FVVIQNEDLGPASPL	986				-0.0025					3755
LERPKTLSP	GATLERPKTLSPGKN	1164									3756
VVQGNLELT	GCQVVQGNLELTYLP	52									3757
LNNTTPVTG	GDPLNNTTPVTGASP	120									3758
LACHQLCAR	GEGLACHQLCARGHC	506									3759
VKIPVAIKV	GENVKIPVAIKVLRE	743	0.0630	0.0047	0.0034	0.0098	-0.0032		-0.0005		3760
LPQPPICTI	GERLPQPPICTIDVY	938	-0.0005				-0.0032				3761
VPIKWMALE	GGKVPIKWMALESIL	881									3762
LIQRNPQLC	GGVLIQRNPQLCYQD	151									3763
WGPGPTQCV	GHCWGPGPTQCVNCS	518									3764
WLGLRSLRE	GISWLGLRSLRELGS	449									3765
LIHHNTHLC	GLALIHHNTHLCFVH	464									3766
ISWLGLRSL	GLGISWLGLRSLREL	447									3767
LALLPPGAA	GLLLALLPPGAASTQ	10									3768
VFDGDLGMG	GSDVFDGDLGMGAAK	1082									3769

TableXIX
HER2/NEU DR Super Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
YNYLSTDVG	ACPYNYLSTDVGSCT						3720
VLRENTSPK	AIKVLRENTSPKANK						3721
LQSLPTHDP	AKGLQSLPTHDPSPL						3722
YDGIPAREI	AKPYDGIPAREIPDL						3723
LDIDETEYH	ARLLDIDETEYHADG	-0.0001	-0.0017	-0.0009			3724
VLVKSPNHV	ARNVLVKSPNHVKIT						3725
LTSHSAVV	ASPLTSHSAVVGIL	0.0480	0.0350	-0.0004			3726
LTLQGLGIS	AYSLTLQGLGISWLG						3727
MAGVGSPYV	AYVMAGVGSPYVSRL						3728
IFGSLAFLP	CKKIFGSLAFLPESF	0.0034	0.0270	-0.0004			3729
FNHSGICEL	CLHFNHSGICELHCP						3730
IAKGMSYLE	CMQIAKGMSYLEDVR						3731
LVTYNTDTF	CPALVTYNTDTFESM						3732
LTRTVCAGG	CQSLTRTVCAGGCAR						3733
LDDKGCPAE	CVDLDDKGCPAEQRA						3734
LGMEHLREV	CYGLGMEHLREVRAV						3735
YVMAGVGSP	DEAYVMAGVGSPYVS	0.0020	0.2000	0.0570			3736
VGEGLACHQ	DECVGEGLACHQLCA						3737
LGMGAAKGL	DGDLGMGAAKGLQSL						3738
VAPLTCSPQ	DGYVAPLTCSPQPEY						3739
LGLEPSEEE	DLTLGLEPSEEEAPR						3740
LRLPASPET	DMKLRLPASPETHLD		-0.0013				3741
FCVARCPSG	DPPFCVARCPSGVKP						3742
FYRSLLEDD	DSTFYRSLLEDDDMG						3743
LVHRDLAAR	DVRLVHRDLAARNVL						3744
MIMVKCWMI	DVYMIMVKCWMIDSE	-0.0003	0.1300	0.0450			3745
VLQGLPREY	ECRVLQGLPREYVNA						3746
YALAVLDNG	EDNYALAVLDNGDPL						3747
LPAARPAGA	EGPLPAARPAGATLE						3748
YTFGASCVT	EGRYTFGASCVTACP						3749
YLPTNASLS	ELTYLPTNASLSFLQ						3750
LRRRFTHQS	ESILRRRFTHQSDVW						3751
LRKVKVLGS	ETELRKVKVLGSGAF	0.0014	0.0380	0.0250			3752
LVEPLTPSG	ETELVEPLTPSGAMP		-0.0013				3753
LVSEFSRMA	FRELVSEFSRMARDP						3754
IQNEDLGPA	FVYIQNEDLGPASPL						3755
LERPKTLSP	GATLERPKTLSPGKN						3756
VVQGNLELT	GCQVVQGNLELTYLP						3757
LNNTTPVTG	GDPLNNTTPVTGASP						3758
LACHQLCAR	GEGLACHQLCARGHC						3759
VKIPVAIKV	GENVKIPVAIKVLRE	0.0004	0.0310	0.0010			3760
LPQPPICTI	GERLPQPPICTIDVY		-0.0011				3761
VPIKWMALE	GGKVPIKWMALESIL						3762
LIQRNPQLC	GGVLIQRNPQLCYQD						3763
WGPGPTQCV	GHCWGPGPTQCVNCS						3764
WLGLRSLRE	GISWLGLRSLRELGS						3765
LIHHNTHLC	GLALIHHNTHLCFVH						3766
ISWLGLRSL	GLGISWLGLRSLREL						3767
LALLPPGAA	GLLLALLPPGAASTQ						3768
VFDGDLGMG	GSDVFDGDLGMGAAK						3769

Table XIX:
HER2/NEU DR Super Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	Position	DR1	DR2w81	DR2w282	DR3	DR4w4	DR4w15	DR5w11	DR5w12	SEQ ID NO.
YVSRLLGIC	GSPYVSRLLGICLTS	778									3770
MKLRLPASP	GTDMKLRLPASPETH	28	0.0010				-0.0025				3771
YKGIWIPDG	GTVYKGIWIPDGENV	732									3772
VWELMTFGA	GVTVWELMTFGAKPY	909	1.4000				0.0330				3773
YISAWPDSL	GYLYISAWPDSLPDL	408									3774
FVHTVPWDQ	HLCFVHTVPWDQLFR	473									3775
VRQVPLQRL	HNQVRQVPLQRLRIV	88									3776
LAARNVLVK	HRDLAARNVLVKSPN	843									3777
ICELHCPAL	HSGICELHCPALVTY	260									3778
ITDFGLARL	HVKITDFGLARLLDI	858									3779
LHCPALVTY	ICELHCPALVTYNTD	263									3780
IDVYMIMVK	ICTIDVYMIMVKCWM	946									3781
LRENTSPKA	IKVLRENTSPKANKE	752	-0.0005				-0.0032				3782
MALESILRR	IKWMALESILRRRFT	886	0.9500				0.0400				3783
VVVLGVVFG	ILLVVVLGVVFGILI	661									3784
VQGYVLIAH	IQEVQGYVLIAHNQV	77									3785
YTMRRLLQE	IRKYTMRRLLQETEL	682									3786
VVGILLVVV	ISAVVGILLVVVLGV	655									3787
WPDSLPDLS	ISAWPDSLPDLSVFQ	412									3788
LGLRSLREL	ISWLGLRSLRELGSG	450									3789
FGLARLLDI	ITDFGLARLLDIDET	861	0.0048				-0.0032				3790
YLYISAWPD	ITGYLYISAWPDSLP	406									3791
MIDSECRPR	KCWMIDSECRPRFRE	957									3792
FAFGGAVEN	KDVFAFGGAVENPEY	1182									3793
LDEAYVMAG	KEILDEAYVMAGVGS	765	0.0036				0.0073				3794
LPTDCCHEQ	KGPLPTDCCHEQCAA	228				-0.0027					3795
VAIKVLREN	KIPVAIKVLRENTSP	747									3796
LSYMPIWKF	KPDLSYMPIWKFPDE	605	0.0330				-0.0025				3797
VLGSGAFGT	KVKVLGSGAFGTVYK	722									3798
IKWMALESI	KVPIKWMALESILRR	883	2.2000	2.7000	2.1000	0.0620	0.0690		0.0073		3799
LCRWGLLLA	LAALCRWGLLLALLP	3									3800
LHFNHSGIC	LACLHFNHSGICELH	253									3801
LPPGAASTQ	LALLPPGAASTQVCT	13									3802
LDNGDPLNN	LAVLDNGDPLNNTTP	114									3803
WGLLLALLP	LCRWGLLLALLPPGA	6	0.0940				-0.0025				3804
VFGILIKRR	LGVVFGILIKRRQQK	667									3805
LLPPGAAST	LLALLPPGAASTQVC	12									3806
ICLTSTVQL	LLGICLTSTVQLVTQ	785									3807
WCMQIAKGM	LLNWCMQIAKGMSYL	822	0.8400	0.0057	1.2000	0.0093	0.0011		0.4000		3808
VVLGVVFGI	LLVVVLGVVFGILIK	662	-0.0008				-0.0025				3809
LGISWLGLR	LQGLGISWLGLRSLR	445									3810
LPREYVNAR	LQGLPREYVNARHCL	547				-0.0027					3811
YSEDPTVPL	LQRYSEDPTVPLPSE	1109				0.0270					3812
LPTHDPSPL	LQSLPTHDPSPLQRY	1098									3813
IRGRILIHG	LQVIRGRILIHGAYS	428									3814
LGSGLALIH	LRELGSGLALIHHNT	458	0.0310				-0.0025				3815
LQLRSLTEI	LRELQLRSLTEILKG	137									3816
VRAVTSANI	LREVRAVTSANIQEF	350									3817
VRGTQLFED	LRIVRGTQLFEDNYA	99									3818
VKVLGSGAF	LRKVKVLGSGAFGTV	720									3819

TableXIX

HER2/NEU DR Super Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DRBw2	DR9	DRw53	SEQ ID NO.
YVSRLLGIC	GSPYVSRLLGICLTS						3770
MKLRLPASP	GTDMKLRLPASPETH		-0.0013				3771
YKGIWIPDG	GTVYKGIWIPDGENV						3772
VWELMTFGA	GVTVWELMTFGAKPY		0.0170				3773
YISAWPDSL	GYLYISAWPDSLPDL						3774
FVHTVPWDQ	HLCFVHTVPWDQLFR						3775
VRQVPLQRL	HNQVRQVPLQRLRIV						3776
LAARNVLVK	HRDLAARNVLVKSPN						3777
ICELHCPAL	HSGICELHCPALVTY						3778
ITDFGLARL	HVKITDFGLARLLDI						3779
LHCPALVTY	ICELHCPALVTYNTD						3780
IDVYMIMVK	ICTIDVYMIMVKCWM						3781
LRENTSPKA	IKVLRENTSPKANKE		-0.0011				3782
MALESILRR	IKWMALESILRRRFT		0.0040				3783
VVVLGVVFG	ILLVVVLGVVFGIEI						3784
VQGYVLIAH	IQEVQGYVLIAHNQV						3785
YTMRRLLQE	IRKYTMRRLLQETEL						3786
VVGILLVVV	ISAVVGILLVVVLGV						3787
WPDSLPDLS	ISAWPDSLPDLSVFQ						3788
LGLRSLREL	ISWLGLRSLRELGSG						3789
FGLARLLDI	ITDFGLARLLDIDET		-0.0011				3790
YLYISAWPD	ITGYLYISAWPDSLP						3791
MIDSECRPR	KCWMIDSECRPRFRE						3792
FAFGGAVEN	KDVFAFGGAVENPEY						3793
LDEAYVMAG	KEILDEAYVMAGVGS		-0.0011				3794
LPTDCCHEQ	KGPLPTDCCHEQCAA						3795
VAIKVLREN	KIPVAIKVLRENTSP						3796
LSYMPIWKF	KPDLSYMPIWKFPDE		0.0029				3797
VLGSGAFGT	KVKVLGSGAFGTVYK						3798
IKWMALESI	KVPIKWMALESILRR	0.0031	0.0190	0.0079			3799
LCRWGLLLA	LAALCRWGLLLALLP						3800
LIIFNHSGIC	LACLIFNHSGICELH						3801
LPPGAASTQ	LALLPPGAASTQVCT						3802
LDNGDPLNN	LAVLDNGDPLNNTTP						3803
WGLLLALLP	LCRWGLLLALLPPGA		0.0021				3804
VFGILIKRR	LGVVFGILIKRRQQK						3805
LLPPGAAST	LLALLPPGAASTQVC						3806
ICLTSTVQL	LLGICLTSTVQLVTQ						3807
WCMQIAKGM	LLNWCMQIAKGMSYL	0.0390	0.1200	0.4100			3808
VVLGVVFGI	LLVVVLGVVFGILIK		0.0019				3809
LGISWLGLR	LQGLGISWLGLRSLR						3810
LPREYVNAR	LQGLPREYVNARHCL						3811
YSEDPTVPL	LQRYSEDPTVPLPSE						3812
LPTHDPSPL	LQSLPTHDPSPLQRY						3813
IRGRILHNG	LQVIRGRILHNGAYS						3814
LGSGLALIH	LRELGSGLALIHHNT		-0.0013				3815
LQLRSLTEI	LRELQLRSLTEILKG						3816
VRAVTSANI	LREVRAVTSANIQEF						3817
VRGTQLFED	LRIVRGTQLFEDNYA						3818
VKVLGSGAF	LRKVKVLGSGAFGTV						3819

Table XIX.

HER2/NEU DR Super Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	Position	DR1	DR2wβ1	DR2w2β2	DR3	DR4w4	DR4w15	DR5w11	DR5w12	SEQ ID NO.
LRELGSGLA	LRSLRELGSGLALIH	455									3820
FQNLQVIRG	LSVFQNLQVIRGRIL	422	0.1800	0.0280	0.0740	0.0010	0.0670		0.0100		3821
ILKGGVLIQ	LTEILKGGVLIQRNP	145									3822
IDTNRSRAC	LTLIDTNRSRACHPC	181									3823
IISAVVGIL	LTSIISAVVGILLVV	651	0.1900				-0.0025				3824
LPTNASLSF	LTYLPTNASLSFLQD	62	0.4900	0.0100	0.0560	0.0150	0.3300		0.0041		3825
WDQDPPERG	LYYWDQDPPERGAPP	1220									3826
VGSPYVSRL	MAGVGSPYVSRLLGI	774									3827
LREVRAVTS	MEIILREVRAVTSANI	347									3828
VKCWMIDSE	MIMVKCWMIDSECRP	953									3829
LKETELRKV	MRILKETELRKVKVL	712									3830
LEDVRLVHR	MSYLEDVRLVHRDLA	833									3831
LGPASPLDS	NEDLGPASPLDSTFY	991									3832
VTCFGPEAD	NGSVTCFGPEADQCV	571									3833
VKDVFAFGG	NGVVKDVFAFGGAVE	1178									3834
LTYLPTNAS	NLELTYLPTNASLSF	59	0.4700	0.0280	0.0090	0.0010	0.3800		0.0050		3835
VIRGRILHN	NLQVIRGRILHNGAY	427									3836
YWDQDPPER	NLYYWDQDPPERGAP	1219									3837
LALTLIDTN	NNQLALTLIDTNRSR	176									3838
LCYQDTILW	NPQLCYQDTILWKDI	158									3839
LCFVITVPW	NTHLCFVITVPWDQL	471									3840
INCTHSCVD	PCPINCTHSCVDLDD	625									3841
LPDLSVFQN	PDSLPDLSVFQNLQV	416									3842
LQVFETLEE	PEQLQVFETLEEITG	394									3843
FDGDPASNT	PESFDGDPASNTAPL	378				-0.0027					3844
VNQPDVRPQ	PEYVNQPDVRPQPPS	1137									3845
VARCPSGVK	PFCVARCPSGVKPDL	594									3846
LRELQLRSL	PGGLRELQLRSLTEI	134									3847
WMALESILR	PIKWMALESILRRRF	885	0.7900				0.0350				3848
VKPDLSYMP	PSGVKPDLSYMPIWK	601				-0.0027					3849
FKGTPTAEN	PSTFKGTPTAENPEY	1234	-0.0005				-0.0032				3850
YLSTDVGSC	PYNYLSTDVGSCTLV	300									3851
ILWKDIFHK	QDTILWKDIFHKNNQ	164									3852
VEECRVLQG	QECVEECRVLQGLPR	538									3853
FCPDPAPGA	QGFFCPDPAPGAGGM	1028	-0.0005				0.0230				3854
LELTYLPTN	QGNLELTYLPTNASL	57									3855
LTLIDTNRS	QLALTLIDTNRSRAC	178									3856
YQDTILWKD	QLCYQDTILWKDIFH	160									3857
VRPQPPSPR	QPDVRPQPPSPREGP	1142	0.0007				-0.0032				3858
ICTIDVYMI	QPPICTIDVYMIMVK	943	0.0670	0.0540	0.0027	0.0976	0.0060		0.0046		3859
FFCPDPAPG	QQGFFCPDPAPGAGG	1027									3860
IRKYTMRRL	QQKIRKYTMRRLLQE	679									3861
VWSYGVTVW	QSDVWSYGVTVWELM	902									3862
LQRLRIVRG	QVPLQRLRIVRGTQL	93									3863
VNARHCLPC	REYVNARHCLPCHPE	552									3864
ILHNGAYSL	RGRILHNGAYSLTLQ	432									3865
LGSQDLLNW	RGRLGSQDLLNWCMQ	814									3866
YQGCQVVQG	RHLYQGCQVVQGNLE	47									3867
FRELVSEFS	RPRFRELVSEFSRMA	966									3868
LQETELVEP	RRLLQETELVEPLTP	688									3869

TableXIX.

HER2/NEU DR Super Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
LRELGSGLA	LRSLRELGSGLALIH						
FQNLQVIRG	LSVFQNLQVIRGRIL	0.0057	0.2900	0.0330			3820
ILKGGVLIQ	LTELKGGVLIQRNP						3821
IDTNRSRAC	LTLIDTNRSRACHPC						3822
IISAVVGIL	LTSIISAVVGILLVV		0.0049				3823
LPTNASLSF	LTYLPTNASLSFLQD	0.0280	0.3200	0.0054			3824
WDQDPPERG	LYYWDQDPPERGAPP						3825
VGSPYVSRL	MAGVGSPYVSRLLGI						3826
LREVRAVTS	MEILREVRAVTSANI						3827
VKCWMIDSE	MIMVKCWMIDSECRP						3828
LKETELRKV	MRILKETELRKVKVL						3829
LEDVRLVHR	MSYLEDVRLVHRDLA						3830
LGPASPLDS	NEDLGPASPLDSTFY						3831
VTCFGPEAD	NGSVTCFGPEADQCV						3832
VKIDVFAFGG	NGVVKIDVFAFGGAVE						3833
LTYLPTNAS	NLELTYLPTNASLSF	0.0017	0.0680	0.0220			3834
VIRGRILHN	NLQVIRGRILHNGAY						3835
YWDQDPPER	NLYYWDQDPPERGAP						3836
LALTLIDTN	NNQLALTLIDTNRSR						3837
LCYQDTILW	NPQLCYQDTILWKDI						3838
LCFVHTVPW	NTHLCFVHTVPWDQL						3839
INCTHSCVD	PCPINCTHSCVDLDD						3840
LPDLSVFQN	PDSLPDLSVFQNLQV						3841
LQVFETLEE	PEQLQVFETLEEITG						3842
FDGDPASNT	PESFDGDPASNTAPL						3843
VNQPDVRPQ	PEYVNQPDVRPQPPS						3844
VARCPSGVK	PFCVARCPSGVKPDL						3845
LRELQLRSL	PGGLRELQLRSLTEI						3846
WMALESILR	PIKWMALESILRRRF		0.0078				3847
VKPDLSYMP	PSGVKPDLSYMPIWK						3848
FKGTPTAEN	PSTFKGTPTAENPEY		-0.0011				3849
YLSTDVGSC	PYNYLSTDVGSCTLV						3850
ILWKDIFHK	QDTILWKDIFHKNNQ						3851
VEECRVLQG	QECVEECRVLQGLPR						3852
FCPDPAPGA	QGFFCPDPAPGAGGM		-0.0011				3853
LELTYLPTN	QGNLELTYLPTNASL						3854
LTLIDTNRS	QLALTLIDTNRSRAC						3855
YQDTILWKD	QLCYQDTILWKDIFH						3856
VRPQPPSPR	QPDVRPQPPSPREGP		-0.0011				3857
ICTIDVYMI	QPPICTIDVYMIMVK	0.0013	0.1000	0.0051			3858
FFCPDPAPG	QQGFFCPDPAPGAGG						3859
IRKYTMRRL	QQKIRKYTMRRLLQE						3860
VWSYGVTVW	QSDVWSYGVTVWELM						3861
LQRLRIVRG	QVPLQRLRIVRGTQL						3862
VNARHCLPC	REYVNARHCLPCHPE						3863
ILHNGAYSL	RGRILHNGAYSLTLQ						3864
LGSQDLLNW	RGRLGSQDLLNWCMQ						3865
YQGCQVVQG	RHLYQGCQVVQGNLE						3866
FRELVSEFS	RPRFRELVSEFSRMA						3867
LQETELVEP	RRLLQETELVEPLTP						3868
							3869

Table XIX:
HER2/NEU DR Super Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	Position	DR1	DR2w81	DR2w2B2	DR3	DR4w4	DR4w15	DR5w11	DR5w12	SEQ ID NO.
LEDDDMGDL	RSLLEDDDMGDLVDA	1006				0.0080					3870
LLLALLPPG	RWGLLLALLPPGAAS	8	12.0000	0.1300	0.0270	0.0010	0.2800		0.0710		3871
FGASCVTAC	RYTFGASCVTACPYN	288									3872
VGILLVVVL	SAVVGILLVVVLGVV	656									3873
WSYGVTVWE	SDVWSYGVTVWELMT	903									3874
LQGLGISWL	SLTLQGLGISWLGLR	442									3875
LLNWCMQIA	SQDLLNWCMQIAKGM	819									3876
LRGQECVEE	SQFLRGQECVEECRV	532									3877
LGICLTSTV	SRLLGICLTSTVQLV	783	0.3500	0.0220	-0.0007	0.0062	0.1200		0.0140		3878
VGSCTLVCP	STDVGSCTLVCPLHN	305									3879
VTVWELMTF	SYGVTVWELMTFGAK	907									3880
LQPEQLQVF	TAPLQPEQLQVFETL	389				0.0023					3881
YVAPLTCSP	TDGYVAPLTCSPQPE	1124	-0.0005				-0.0032				3882
LKGGVLIQR	TEILKGGVLIQRNPQ	146									3883
VEPLTPSGA	TELVEPLTPSGAMPN	694									3884
VYMIMVKCW	TIDVYMIMVKCWMID	948									3885
FEDNYALAV	TQLFEDNYALAVLDN	105	0.0530				-0.0025				3886
MPYGCLLDH	TQLMPYGCLLDHVRE	798									3887
VCAGGCARC	TRTVCAGGCARCKGP	216									3888
VTGASPGGL	TTPVTGASPGGLREL	126									3889
LVTQLMPYG	TVQLVTQLMPYGCLL	793	0.2300	0.7500	0.0009	0.0010	0.0460		-0.0005		3890
LHNQEVTAE	VCPLHNQEVTAEDGT	314	-0.0004				-0.0025				3891
LTPSGAMPN	VEPLTPSGAMPNQAQ	697	-0.0008				-0.0025				3892
LLVVVLGVV	VGILLVVVLGVVFGI	659									3893
VPWDQLFRN	VHTVPWDQLFRNPHQ	477				0.0220					3894
VVFGILIKR	VLGVVFGILIKRRQQ	666	0.0700	0.0110	0.0620	0.0021	0.0029		0.4700		3895
VTQLMPYGC	VQLVTQLMPYGCLLD	794									3896
VTSANIQEF	VRAVTSANIQEFAGC	353									3897
VHRDLAARN	VRLVHRDLAARNVLV	839	0.0340	0.0064	0.0033	0.3400	0.0150		0.2700		3898
VPLQRLRIV	VRQVPLQRLRIVRGT	91									3899
LLGICLTST	VSRLLGICLTSTVQL	782									3900
LMPYGCLLD	VTQLMPYGCLLDHVR	797									3901
ILLVVVLGV	VVGILLVVVLGVVFG	658	-0.0004				-0.0025				3902
LMTFGAKPY	VWELMTFGAKPYDGI	912	0.0870	0.0990	0.1000	0.0010	0.0550		0.0054		3903
LLALLPPGA	WGLLLALLPPGAAST	9	5.1000	0.2100	0.0110	0.0013	1.3000		0.2500		3904
IPAREIPDL	YDGIPAREIPDLLEK	923									3905
MVKCWMIDS	YMIMVKCWMIDSECR	952									3906
IAHNQVRQV	YVLIAHNQVRQVPLQ	83									3907

TableXIX
HER2/NEU DR Super Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
LEDDDMGDL	RSLLEDDDMGDLVDA						3870
LLLALLPPG	RWGLLLALLPPGAAS	-0.0003	-0.0013	0.1200			3871
FGASCVTAC	RYTFGASCVTACPYN						3872
VGILLVVVL	SAVVGILLVVVLGVV						3873
WSYGVTVWE	SDVWSYGVTVWELMT						3874
LQGLGISWL	SLTLQGLGISWLGLR						3875
LLNWCMQIA	SQDLLNWCMQIAKGM						3876
LRGQECVEE	SQFLRGQECVEECRV						3877
LGICLTSTV	SRLLGICLTSTVQLV	0.3400	0.5600	0.0009			3878
VGSCTLVCP	STDVGSCTLVCPLHN						3879
VTVWELMTF	SYGVTVWELMTFGAK						3880
LQPEQLQVF	TAPLQPEQLQVFETL						3881
YVAPLTCSP	TDGYVAPLTCSPQPE		-0.0011				3882
LKGGVLIQR	TELKGGVLIQRNPQ						3883
VEPLTPSGA	TELVEPLTPSGAMPN						3884
VYMIMVKCW	TIDVYMIMVKCWMID						3885
FEDNYALAV	TQLFEDNYALAVLDN		0.0160				3886
MPYGCLLDH	TQLMPYGCLLDHVRE						3887
VCAGGCARC	TRTVCAGGCARCKGP						3888
VTGASPGGL	TTPVTGASPGGLREL						3889
LVTQLMPYG	TVQLVTQLMPYGCLL	0.0031	0.0100	0.0069			3890
LHNQEVTAE	VCPLHNQEVTAEDGT		-0.0013				3891
LTPSGAMPN	VEPLTPSGAMPNQAQ		-0.0011				3892
LLVVVLGVV	VGILLVVVLGVVFGI						3893
VPWDQLFRN	VHTVPWDQLFRNPHQ						3894
VVFGILIKR	VLGVVFGILIKRRQQ	0.0150	0.0320	0.6400			3895
VTQLMPYGC	VQLVTQLMPYGCLLD						3896
VTSANIQEF	VRAVTSANIQEFAGC						3897
VHRDLAARN	VRLVHRDLAARNVLV	0.0430	0.0230	0.1000			3898
VPLQRLRIV	VRQVPLQRLRIVRGT						3899
LLGICLTST	VSRLLGICLTSTVQL						3900
LMPYGCLLD	VTQLMPYGCLLDHVR						3901
ILLVVVLGV	VVGILLVVVLGVVFG		-0.0013				3902
LMTFGAKPY	VWELMTFGAKPYDGI	0.0004	0.0370	0.0089			3903
LLALLPPGA	WGLLLALLPPGAAST	-0.0003	-0.0013	0.4500			3904
IPAREIPDL	YDGIPAREIPDLLEK						3905
MVKCWMIDS	YMIMVKCWMIDSECR						3906
IAHNQVRQV	YVLIAHNQVRQVPLQ						3907

Table XXa
HER2/NEU DR 3a Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	Position	DR1	DR2w2β1	DR2w2β2	DR3	DR4w4	DR4w15	DR5w11	DR5w12	SEQ ID NO.
VLRENTSPK	AIKVLRENTSPKANK	751				0.0075					3908
LDIDETEYH	ARLLDIDETEYHADG	867	0.0001	-0.0006	-0.0007	0.3100	-0.0055		-0.0008		3909
LGMEHLREV	CYGLGMEHLREVRAV	342				0.0083					3910
LGLEPSEEE	DLTLGLEPSEEEAPR	1058				-0.0025					3911
YYWDQDPPE	DNLYYWDQDPPERGA	1218				-0.0025					3912
LWKDIFHKN	DTILWKDIFHKNNQL	165				-0.0027					3913
YHADGGKVP	ETEYHADGGKVPIKW	874				-0.0027					3914
LVSEFSRMA	FRELVSEFSRMARDP	969				0.0710					3915
MARDPQRFV	FSRMARDPQRFVVIQ	976				0.1600					3916
IQNEDLGPA	FVVIQNEDLGPASPL	986				-0.0025					3917
VDAEEYLVP	GDLVDAEEYLVPQQG	1015				0.0250					3918
LFEDNYALA	GTQLFEDNYALAVLD	104				0.2200					3919
MALESILRR	IKWMALESILRRRFT	886	0.9500				0.0400				3920
FPDEEGACQ	IWKFPDEEGACQPCP	613				-0.0027					3921
LPTDCCHEQ	KGPLPTDCCHEQCAA	228				-0.0027					3922
VVKDVFAFG	KNGVVKDVFAFGGAV	1177				-0.0025					3923
LPREYVNAR	LQGLPREYVNARHCL	547				-0.0027					3924
YSEDPTVPL	LQRYSEDPTVPLPSE	1109				0.0270					3925
YNTDTFESM	LVTYNTDTFESMPNP	271				-0.0027					3926
LLQETELVE	MRRLLQETELVEPLT	687				-0.0027					3927
HLDEAYVMA	NKEILDEAYVMAGVG	764				0.0047					3928
VTAEDGTQR	NQEVTAEDGTQRCEK	319				-0.0027					3929
FDGDPASNT	PESFDGDPASNTAPL	378				-0.0027					3930
VKPDLSYMP	PSGVKPDLSYMPIWK	601				-0.0027					3931
FCPDPAPGA	QGFFCPDPAPGAGGM	1028	-0.0005				0.0230				3932
ILKETELRK	QMRILKETELRKVKV	711	0.0419	0.0150	0.5900	0.3200	-0.0055		0.0041		3933
LEDDDMGDL	RSLLEDDDMGDLVDA	1006				0.0080					3934
FDGDLGMGA	SDVFDGDLGMGAAKG	1083				-0.0025					3935
FLPESFDGD	SLAFLPESFDGDPAS	373				-0.0027					3936
FLQDIQEVQ	SLSFLQDIQEVQGYV	70				0.0520					3937
LQPEQLQVF	TAPLQPEQLQVFETL	389				0.0023					3938
LPSETDGYV	TVPLPSETDGYVAPL	1117				-0.0025					3939
VPWDQLFRN	VHTVPWDQLFRNPHQ	477				0.0220					3940
VHRDLAARN	VRLVHRDLAARNVLV	839	0.0340	0.0064	0.0033	0.3400	0.0150		0.2700		3941
FGPEADQCV	VTCFGPEADQCVACA	574				-0.0027					3942
LSTDVGSCT	YNYLSTDVGSCTLVC	301				0.0059					3943
LLEDDDMGD	YRSLLEDDDMGDLVD	1005				0.0630					3944

Table XXa
HER2/NEU DR 3a Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
VLRENTSPK	AIKVLRENTSPKANK						3908
LDIDETEYH	ARLLDIDETEYHADG	-0.0001	-0.0017	-0.0009			3909
LGMEHLREV	CYGLGMEHLREVRAV						3910
LGLEPSEEE	DLTLGLEPSEEEAPR						3911
YYWDQDPPE	DNLYYWDQDPPERGA						3912
LWKDIFHKN	DTILWKDIFHKNNQL						3913
YHADGGKVP	ETEYHADGGKVPIKW						3914
LVSEFSRMA	FRELVSEFSRMARDP						3915
MARDPQRFV	FSRMARDPQRFVVIQ						3916
IQNEDLGPA	FVVIQNEDLGPASPL						3917
VDAEEYLVP	GDLVDAEEYLVPQQG						3918
LFEDNYALA	GTQLFEDNYALAVLD						3919
MALESILRR	IKWMALESILRRRFT		0.0040				3920
FPDEEGACQ	IWKFPDEEGACQPCP						3921
LPTDCCHEQ	KGPLPTDCCHEQCAA						3922
VVKDVFAFG	KNGVVKDVFAFGGAV						3923
LPREYVNAR	LQGLPREYVNARHCL						3924
YSEDPTVPL	LQRYSEDPTVPLPSE						3925
YNTDTFESM	LVTYNTDTFESMPNP						3926
LLQETELVE	MRRLLQETELVEPLT						3927
ILDEAYVMA	NKEILDEAYVMAGVG						3928
VTAEDGTQR	NQEVTAEDGTQRCEK						3929
FDGDPASNT	PESFDGDPASNTAPL						3930
VKPDLSYMP	PSGVKPDLSYMPIWK						3931
FCPDPAPGA	QGFFCPDPAPGAGGM		-0.0011				3932
ILKETELRK	QMRILKETELRKVKV	0.0008	0.0130	0.0064			3933
LEDDDMGDL	RSLLEDDDMGDLVDA						3934
FDGDLGMGA	SDVFDGDLGMGAAKG						3935
FLPESFDGD	SLAFLPESFDGDPAS						3936
FLQDIQEVQ	SLSFLQDIQEVQGYV						3937
LQFEQLQVF	TAPLQPEQLQVFETL						3938
LPSETDGYV	TVPLPSETDGYVAPL						3939
VPWDQLFRN	VIITVPWDQLFRNPHQ						3940
VHRDLAARN	VRLVHRDLAARNVLV	0.0430	0.0230	0.1000			3941
FGPEADQCV	VTCFGPEADQCVACA						3942
LSTDVGSCT	YNYLSTDVGSCTLVC						3943
LLEDDDMGD	YRSLLEDDDMGDLVD						3944

Table XXb
HER2/NEU DR 3b Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	Position	DR1	DR2w201	DR2w202	DR3	DR4w4	DR4w15	DR5w11	DR5w12	SEQ ID NO.
LIDTNRSRA	ALTLIDTNRSRACHP	180				0.0350					3945
IDSECRPRF	CWMIDSECRPRFREL	958	0.0036	-0.0006	0.0150	0.4500	-0.0055		-0.0008		3946
YLEDVRLVH	GMSYLEDVRLVHRDL	832				0.1800					3947
VDLDDKGCP	HSCVDLDDKGCPAEQ	632				-0.0027					3948
HHHNTHLCF	LALHHHNTHLCFVHT	465	0.0140	0.0990	0.0009	0.3100	-0.0055		0.0025		3949
AAPQPHPPP	QGGAAPQPHPPPAFS	1200				-0.0025					3950
ASPETHLDM	RLPASPETHLDMLRH	34				-0.0027					3951
AHNQVRQVP	VLAHHNQVRQVPLQR	84				0.0290					3952
LFRNPHQAL	WDQLFRNPHQALLHT	482	-0.0001	0.0015	-0.0007	0.9000	-0.0055		-0.0008		3953

Table XXb
HER2/NEU DR 3b Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
LIDTNRSRA	ALTLIDTNRSRACHP						
IDSECRPRF	CWMIDSECRPRFREL	-0.0001	-0.0014	0.0028			3945
YLEDVRLVH	GMSYLEDVRLVHRDL						3946
VDLDDKGCP	HSCVDLDDKGCPAEQ						3947
HHHNTHLCF	LALHHHNTHLCFVHT	0.7500	0.0200	0.0330			3948
AAPQPHPPP	QGGAAPQPHPPPAFS						3949
ASPETHLDM	RLPASPETHLDMLRH						3950
AHNQVRQVP	VLIAHNQVRQVPLQR						3951
LFRNPHQAL	WDQLFRNPHQALLHT	0.0410	-0.0017	-0.0009			3952
							3953

TABLE XXI. Population coverage with combined HLA Supertypes

HLA-SUPERTYPES	PHENOTYPIC FREQUENCY					
	Caucasian	North American Black	Japanese	Chinese	Hispanic	Average
a. Individual Supertypes						
A2	45.8	39.0	42.4	45.9	43.0	43.2
A3	37.5	42.1	45.8	52.7	43.1	44.2
B7	43.2	55.1	57.1	43.0	49.3	49.5
A1	47.1	16.1	21.8	14.7	26.3	25.2
A24	23.9	38.9	58.6	40.1	38.3	40.0
B44	43.0	21.2	42.9	39.1	39.0	37.0
B27	28.4	26.1	13.3	13.9	35.3	23.4
B62	12.6	4.8	36.5	25.4	11.1	18.1
B58	10.0	25.1	1.6	9.0	5.9	10.3
b. Combined Supertypes						
A2, A3, B7	84.3	86.8	89.5	89.8	86.8	87.4
A2, A3, B7, A24, B44, A1	99.5	98.1	100.0	99.5	99.4	99.3
A2, A3, B7, A24, B44, A1, B27, B62, B58	99.9	99.6	100.0	99.8	99.9	99.8

Table XXII. Crossbinding data of A2 supermotif peptides

Source	AA	Sequence	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	No. A2 Alleles Crossbound
Her2/neu.5	9	ALCRWGLLL	100	--	278	--	--	2
Her2/neu.5	10	ALCRWGLLLA	139	1955	12	1947	2500	2
Her2/neu.48	9	HLYQGCQVV	139	307	13	514	1143	3
Her2/neu.106	9	QLFEDNYAL	17	226	11	463	2105	4
Her2/neu.106	10	QLFEDNYALA	357	662	9.1	218	74	4
Her2/neu.144	10	SLTEILKGGV	238	--	22	--	--	2
Her2/neu.153	9	VLIQRNPQL	23	3909	3.3	1057	--	2
Her2/neu.369	9	KIFGSLAFL	36	9.0	19	23	3333	4
Her2/neu.435	9	ILHNGAYSL	75	358	100	569	--	3
Her2/neu.466	9	ALIHHNTHL	278	1265	10	1762	--	2
Her2/neu.508	9	GLACHQLCA	417	--	127	--	9091	2
Her2/neu.653	9	SIISAVVGI	69	524	35	285	148	4
Her2/neu.665	9	VVLGVVFGI	14	--	2500	430	2000	2
Her2/neu.689	9	RLLQETELV	21	--	625	34	--	2
Her2/neu.767	9	ILDEAYVMA	238	--	4167	3083	--	1
Her2/neu.773	10	VMAGVGSPYV	200	391	13	3700	--	3
Her2/neu.789	9	CLTSTVQLV	208	457	6.7	308	8000	4
Her2/neu.799	9	QLMPYGCLL	217	977	114	712	--	2
Her2/neu.952	10	YMIMVKCWMI	20	307	83	116	267	5
Her2/neu.952	9	YMIMVKCWM	217	--	625	2643	1000	1

-- indicates binding affinity =10,000nM.

Table XXII. A2 supermotif analog peptides

Source	AA	Sequence	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	No. A2 Alleles Crossbound
Her2/neu.5	9	ALCRWGLLL	100	--	278	--	--	2
Her2/neu.5.T2B3V9	9	ATBRWGLLV	16667	215	323	2177	1739	2
Her2/neu.5V2B3V9	9	AVBRWGLLV	10000	215	141	2177	4706	2
Her2/neu.5.B3	9	ALBRWGLLL	238	1	12	6167	7273	3
Her2/neu.5B3V9	9	ALBRWGLLV	18	33	4.2	285	--	4
Her2/neu.5M2B3V9	9	AMBRWGLLV	36	473	16	726	--	3
Her2/neu.153	9	VLIQRNPQL	23	3909	3.3	1057	--	2
Her2/neu.153V9	9	VLIQRNPQV	55	768	135	385	--	3
Her2/neu.369	9	KIFGSLAFL	36	9.0	19	23	3333	4
Her2/neu.369V2V9	9	KVFGSLAFV	20	19.0	769	15	29	4
Her2/neu.369T2V9	9	KTFGSLAFV	35	13.0	1010	14	17	4
Her2/neu.369L2V9	9	KLFGSLAFV	5.8	7.5	19	17	1270	4
Her2/neu.653	9	SIISAVVGI	69	524	35	285	148	4
Her2/neu.653.L2V9	9	SLISAVVGV	7.1	10	16	20	110	5
Her2/neu.665	9	VVLGVVFGI	14	--	2500	430	2000	2
Her2/neu.665V2V9	9	VVLGVVFGV						
Her2/neu.665L2V9	9	VLLGVVFGV	2.4	17	14	6.0	8000	4
Her2/neu.952	10	YMIMVKCWMI	20	307	83	116	267	5
Her2/neu.952L2V10	10	YLIMVKCWMV	13	56	116	18	84	5
Her2/neu.952L2B7V10	10	YLIMVKBWMV	7.2	66	77	11	851	4

-- indicates binding affinity =10,000nM.

Table XXIII. HLA-A3 Supermotif-bearing Peptides

AA	Sequence	Source	A*0301 nM	A*1101 nM	A*3101 nM	A*3301 nM	A*6801 nM	No. of A3 Alleles Crossbound	CTL Wildtype	CTL Tumor	Published CTL Wildtype	Published CTL Tumor
10	QLRSLTEILK	Her2/neu.141	55	462	667	6170	--	2				
10	ILKGGVLIQR	Her2/neu.148	275	--	25	121	205	4				
10	IVKGGVLIQR	Her2/neu.148.V2	275	7500	72	126	29	4				
10	IVKGGVLIQK	Her2/neu.148.V2K10	26	102	450	6591	27	4				
10	TILWKDIFHK	Her2/neu.166	256	1.7	487	690	200	4				
10	TVLWKDIFHR	Her2/neu.166.V2R10	8462	286	600	76	42	3				
9	ILWKDIFHK	Her2/neu.167	39	19	82	967	1739	3				
9	IVWKDIFHK	Her2/neu.167.V2	23	40	247	853	178	4				
9	IVWKDIFHR	Her2/neu.167.V2R9	143	286	6.0	16	15	5				
10	RTVCAGGCAR	Her2/neu.217	1618	462	40	1318	320	3				
9	TVCAGGCAR	Her2/neu.218	--	261	129	326	83	4				
9	TVBAGGBAR	Her2/neu.218.B3B7	314	111	247	242	8.0	5				
9	TVBAGGBAK	Her2/neu.218.B3B7K9	24	29	--	--	7.3	3				
10	IVWLGLRSLR	Her2/neu.450.V2	234	1936	11	193	7.3	4				
10	IVWLGLRSLK	Her2/neu.450.V2K10	3.9	128	273	2071	12	4				
10	ISWLGLRSLR	Her2/neu.450	268	2222	6.9	223	73	4				
10	HTVPWDQLFR	Her2/neu.478	3143	83	19	88	4.0	4				
10	HVVPWDQLFR	Her2/neu.478.V2	7333	1333	391	193	3.6	3				
10	HVVPWDQLFK	Her2/neu.478.V2K10	180	375	--	--	8.9	3				
9	CVNCSQFLR	Her2/neu.528	7333	194	34	50	18	4				
9	BVNBSQFLR	Her2/neu.528.B1B4	177	80	38	58	10	5				
9	BVNBSQFLK	Her2/neu.528.B1B4K9	34	22	60	4265	15	4				
10	GVVFGILIKR	Her2/neu.668	611	182	305	2071	19	3				
9	VVFGILIKR	Her2/neu.669	100	8.3	13	78	4.0	5	1/3	0/1		
10	VVFGILIKRR	Her2/neu.669	3667	375	290	193	15	4				

-- indicates binding affinity >10,000nM.

Table XXIII. HLA-A3 Supermotif-bearing Peptides

AA	Sequence	Source	A*0301 nM	A*1101 nM	A*3101 nM	A*3301 nM	A*6801 nM	No. of A3 Alleles Crossbound	CTL Wildtype	CTL Tumor	Published CTL Wildtype	Published CTL Tumor
9	VVFGILIKK	Her2/neu.669.K9	22	19	3750	--	35	3				
9	KIRKYTMRR	Her2/neu.681	15	--	16	4028	--	2	3/3	2/3	+ [1]	+
9	VLRENTSPK	Her2/neu.754	28	462	129	290	--	4	4/7	3/6	+ [1]	+
9	VVRENTSPR	Her2/neu.754.V2R9	200	5455	375	126	178	4				
9	LLDHVRENR	Her2/neu.806	297	--	500	326	5714	3				
9	LVARNVLVK	Her2/neu.846.V2	42	214	9000	--	205	3				
9	LAARNVLVK	Her2/neu.846	190	211	--	--	500	3				
9	LVKSPNHVK	Her2/neu.852	23	86	182	784	73	4			+ [1]	
9	LVKSPNHVR	Her2/neu.852.R9	7857	--	198	107	50	3				
9	KITDFGLAR	Her2/neu.860	65	25	100	--	1633	3				
9	KVTDFGLAR	Her2/neu.860.V2	201	76	106	--	133	4				
9	MVLESILRR	Her2/neu.889.V2	216	273	207	153	22	5				
9	MVLESILRK	Her2/neu.889.V2K9	61	16	--	2636	381	3				
9	MALESILRR	Her2/neu.889	3235	253	192	132	127	4				
10	LVSEFSRMAR	Her2/neu.972	1528	182	49	126	36	4				
10	LVSEFSRMAK	Her2/neu.972.K10	250	71	2250	5273	62	3				
10	AVPLDSTFYR	Her2/neu.997.V2	-110000	88	30000	2636	73	2				
10	AVPLDSTFYK	Her2/neU.997.V2K10	550	33	1500	22308	229	2				
10	ASPLDSTFYR	Her2/neu.997	--	90	150	2071	154	3				

1) Kawashima et al., Cancer Research 59:431, 1999

-- indicates binding affinity >10,000nM.

Table XXIV. B7 Supermotif Peptides

AA	Sequence	Source	B*0702 nM	B*3501 nM	B*5101 nM	B*5301 nM	B*5401 nM	No. of B7 Alleles Crossbound
9	LPTNASLSF	Her2/neu.65	212	114	809	34	--	3
10	LPTNASLSFL	Her2/neu.65	290	--	2500	--	--	1
9	FPTNASLSF	Her2/neu.65.F1	1.1	7.9	85	6.2	556	4
9	FPTNASLSI	Her2/neu.65.F1I9	4.6	300	8.7	14	2.8	5
10	FPTNASLSFI	Her2/neu.65.F1I10	120	424	23	85	53	5
10	FPTNASLSFL	Her2/neu.65.F1	18	200	262	172	119	5
9	FPLNNTTPI	Her2/neu.121.F1I9	1.6	200	13	52	1.6	5
9	FPLNNTTPV	Her2/neu.121.F1	0.20	40	7.1	1069	1.1	4
10	SPGGLRELQL	Her2/neu.133	95	--	--	--	--	1
8	SPGGLREL	Her2/neu.133	100	--	--	--	--	1
10	FPGGLRELQI	Her2/neu.133.F1I10	306	--	1774	310	476	3
10	FPMCKGSRCI	Her2/neu.196.F1	183	--	500	6643	303	3
10	MPNPEGRYTI	Her2/neu.282.I10	34	111	13	9.3	46	5
10	FPNPEGRYTI	Her2/neu.282.F1	324	24	9.3	6.6	3.2	5
10	CPLHNQEVTI	Her2/neu.315.I10	458	--	42	274	556	3
10	KPCARVCYGL	Her2/neu.336	149	--	--	--	--	1
10	FPCARVCYGL	Her2/neu.336.F1	190	450	1000	1691	36	3
8	WPDSLPDI	Her2/neu.415.I8	71	--	5000	--	--	1
10	FPHQALLHTA	Her2/neu.488.F1	393	379	4231	--	1.3	3
10	FPHQALLHTI	Her2/neu.488.F1I10	86	655	19	42	4.0	4
11	CPSGVKPDLSY	Her2/neu.600	183	3600	--	--	--	1
11	CPSGVKPDLSI	Her2/neu.600.I11	290	--	138	344	625	3
9	FPSGVKPDI	Her2/neu.600.F1I9	6.3	9000	204	930	44	3
11	FPSGVKPDLSI	Her2/neu.600.F1I11	196	3273	153	211	208	4
9	KPDLSYMPI	Her2/neu.605	76	--	2500	--	--	1
9	FPDLSYMPI	Her2/neu.605.F1	22	167	10	72	31	5

Table XXIV. B7 Supermotif Peptides

AA	Sequence	Source	B*0702 nM	B*3501 nM	B*5101 nM	B*5301 nM	B*5401 nM	No. of B7 Alleles Crossbound
10	CPAEQRASPL	Her2/neu.642	37	--	--	--	--	1
10	FPAEQRASPI	Her2/neu.642.F1I10	3.1	--	98	4895	56	3
10	FPAEQRASPL	Her2/neu.642.F1	1.4	248	859	8455	286	3
10	SPLTSIISAV	Her2/neu.649	61	--	--	--	667	1
9	SPLTSIISI	Her2/neu.649.I9	86	--	275	3444	217	3
9	FPLTSIISA	Her2/neu.649.F1	290	21	663	3000	1.4	3
9	FPLTSIISI	Her2/neu.649.F1I9	212	118	13	28	1.5	5
10	FPLTSIISAI	Her2/neu.649.F1I10	229	327	26	194	3.3	5
10	FPLTSIISAV	Her2/neu.649.F1	220	300	63	2906	1.2	4
11	FPLTSIISAVI	Her2/neu.649.F1I11	367	96	4.6	66	2.2	5
9	FPLTPSGAI	Her2/neu.698.F1I9	0.90	2057	9.2	1632	1.9	3
9	FPLTPSGAM	Her2/neu.698.F1	2.0	16	71	1525	91	4
10	FPSGAMPNQI	Her2/neu.701.F1	229	6546	131	775	100	3
11	MPNQAQMRILI	Her2/neu.706.I11	344	1800	37	12	303	4
9	FPNQAQMRI	Her2/neu.706.F1	68	108	12	18	4.5	5
10	FPNQAQMRII	Her2/neu.706.F1I10	290	514	76	490	59	4
10	FPNQAQMRIL	Her2/neu.706.F1	81	200	458	443	31	5
8	FPKANKEI	Her2/neu.760F1	0.16	--	2500	--	3125	1
9	FPKANKEII	Her2/neu.760.F1I9	8.5	--	55	7154	39	3
9	FPKANKEIL	Her2/neu.760.F1	6.7	4500	190	--	77	3
8	FPYVSRLL	Her2/neu.779.F1	61	600	6.9	581	172	3
10	FPYVSRLLGI	Her2/neu.779.F1	112	3600	7.9	358	9.1	4
11	MPYGCLLDHVI	Her2/neu.801.I11	66	4.2	2.4	1.9	7.7	5
8	FPIKWMAI	Her2/neu.884.F1I8	22	1143	122	930	33	3
8	FPIKWMAL	Her2/neu.884.F1	0.60	248	306	547	40	4
8	KPYDGIPA	Her2/neu.921	367	--	--	490	29	3

Table XXIV. B7 Supermotif Peptides

AA	Sequence	Source	B*0702 nM	B*3501 nM	B*5101 nM	B*5301 nM	B*5401 nM	No. of B7 Alleles Crossbound
8	KPYDGIPI	Her2/neu.921.I8	115	--	74	5167	303	3
8	FPYDGIPA	Her2/neu.921.F1	423	206	157	6200	1.0	4
8	FPYDGIPI	Her2/neu.921.F1I8	177	379	10	344	56	5
11	FPYDGIPAREI	Her2/neu.921.F1I11	141	2880	50	465	15	4
9	LPQPPICTI	Her2/neu.941	196	--	4.2	28	.19	4
9	FPQPPICTI	Her2/neu.941.F1	20	360	17	11	1.7	5
11	FPRFRELVSEI	Her2/neu.966.F1I11	229	240	167	127	28	5
10	FPASPLDSTF	Her2/neu.995.F1	3.7	10	579	10	200	4
10	FPASPLDSTI	Her2/neu.995.F1I10	20	514	20	30	14	5
11	FPLDSTFYRSI	Her2/neu.998.F1I11	229	2667	46	620	2.4	3
11	FPLDSTFYRSL	Her2/neu.998.F1	42	400	324	1192	2.7	4
9	FPLAPSEGI	Her2/neu.1073.F1I9	100	554	204	239	5.9	4
9	FPTHDPSPI	Her2/neu.1101.F1I9	108	600	56	274	50	4
9	FPTHDPSPL	Her2/neu.1101.F1	10	72	3438	1632	208	3
9	FPSETDGYI	Her2/neu.1120.F1I9	220	655	31	37	156	4
9	FPSETDGYV	Her2/neu.1120.F1	204	809	32	517	26	3
11	PPSPREGPLPI	Her2/neu.1149.I11	22	--	423	85	--	3
11	FPSPREGPLPI	Her2/neu.1149.F1I11	190	--	262	5167	263	3
9	FPREGPLPI	Her2/neu.1151.F1I9	0.30	--	14	2818	4.0	3
10	FPREGPLPAI	Her2/neu.1151.F1I10	20	7200	20	620	3.4	3
9	FPLPAARPA	Her2/neu.1155.F1	34	277	1897	--	1.5	3
9	FPLPAARPI	Her2/neu.1155.F1I9	6.5	600	7.1	282	3.8	4
9	FPAARPAGI	Her2/neu.1157.F1I9	9.3	--	131	4227	48	3
11	FPAARPAGATI	Her2/neu.1157.F1I11	39	4235	13	332	50	4
11	FPAARPAGATL	Her2/neu.1157.F1	3.9	360	239	2906	133	4
8	FPGKNGVI	Her2/neu.1174.F1I8	458	--	177	--	385	3

Table XXV. HLA-A1 Motif-Bearing Peptides

AA	Sequence	Source	A*0101 nM
11	GTDMKLRLPY	Her2/neu.28.Y10	50
11	ETHLDMLRHLY	Her2/neu.40	89
9	HLDMLRHLY	Her2/neu.42	2.7
9	HTDMLRHLY	Her2/neu.42.T2	1.9
9	GTQLFEDNY	Her2/neu.104	139
9	GTDLFEDNY	Her2/neu.104.D3	0.90
10	PTDCCHEQCA	Her2/neu.232	125
10	PTDCCHEQCY	Her2/neu.232.Y10	46
11	PTDCCHEQCAA	Her2/neu.232	58
11	PTDCCHEQCAY	Her2/neu.232.Y11	18
10	ESMPNPEGRY	Her2/neu.280	139
10	ETMPNPEGRY	Her2/neu.280.T2	3.9
9	ASCVTACPY	Her2/neu.293	455
11	ASCVTACPYNY	Her2/neu.293	132
9	ATCVTACPY	Her2/neu.293.T2	49
8	VTACPYNY	Her2/neu.296	250
11	VFETLEEITGY	Her2/neu.399	5556
11	ETLEEITGYLY	Her2/neu.401	57
9	ETDEEITGY	Her2/neu.401.D3	17
10	TLEEITGYLY	Her2/neu.402	23
10	TLDEITGYLY	Her2/neu.402.D3	3.4
11	EADQCVACAHY	Her2/neu.580	250
9	VMDGVGSPY	Her2/neu.773.D3	40
10	CMQIAKGMSY	Her2/neu.826	83
10	CTQIAKGMSY	Her2/neu.826.T2	19
9	LLDIDETEY	Her2/neu.869	3.3
9	LTDIDETEY	Her2/neu.869.T2	5.7
10	FTHQSDVWSY	Her2/neu.899	9.3
10	FTDQSDVWSY	Her2/neu.899.D3	0.60
10	PADPLDSTFY	Her2/neu.996.D3	19
9	ATPLDSTFY	Her2/neu.997.T2	36
10	MTDLVDAEEY	Her2/neu.1014.T2	2.3
9	LTCSPQPEY	Her2/neu.1131	192
9	LTDSPQPEY	Her2/neu.1131.D3	32
10	FSPAFDNLYY	Her2/neu.1213	4.5
10	FTPAFDNLYY	Her2/neu.1213.T2	0.80
9	SPDFDNLYY	Her2/neu1214.D3	73.50
10	GTPTAENPEY	Her2/neu.1239	397
10	GTDTAENPEY	Her2/neu.1239.D3	26

Table XXVI. HLA-A24 Motif-Bearing Peptides

AA	Sequence	Source	A*2402 nM
8	RWGLLLAL	Her2/neu.8	480
9	RWGLLLALL	Her2/neu.8	9.2
9	RYGLLALF	Her2/neu8.Y2F9	1.3
9	TYLPTNASL	Her2/neu.63	316
11	TYLPTNASLSF	Her2/neu.63	1.3
9	TYLPTNASF	Her2/neu.63.F9	44
9	CYGLGMEHF	Her2/neu.342.F9	164
10	LYISAWPDSL	Her2/neu.410	143
10	LYISAWPDSF	Her2/neu.410.F10	10
9	AYPDSLPDF	Herw/neu414.Y2F9	24
9	AYSLTLQGL	Her2/neu.440	92
9	AYSLTLQGF	Her2/neu.440.F9	52
9	EYVNARHCF	Her2/neu.553.F9	150
8	SYMPIWKF	Her2/neu.609	38
9	PYVSRLLGI	Her2/neu.780	71
11	PYVSRLLGICL	Her2/neu.780	375
9	PYVSRLLGF	Her2/neu.780.F9	9.2
10	GYSYLEDVRF	Her2/neu.832.Y2F10	235.0
9	KYMALESIF	Her2/neu.887.Y2F9	19.0
9	RYTHQSDVF	Her2/neu.898.Y2F9	60.0
9	VWSYGVTVW	Her2/neu.905	150
9	VYSYGVTVF	Her2/neu.905.Y2F9	16
11	VWSYGVTVWEL	Her2/neu.905	130
9	SYGVTVWEL	Her2/neu.907	100
9	SYGVTVWEF	Her2/neu.907.F9	26
9	VYMIMVKCW	Her2/neu.951	75
11	VYMIMVKCWMI	Her2/neu.951	6.7
9	VYMIMVKCF	Her2/neu.951.F9	19
9	RYRELVSEF	Her2/neu.968.Y2	36
9	RYARDPQRF	Her2/neu.978.Y2	120

Table XXVII. HLA-A2 Supermotif-bearing Peptides

AA	Sequence	Source	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	No. of A2 Alleles Crossbound	CTL Wildtype [1]	CTL Tumor [1]	CTL Wildtype [2]	CTL Tumor [2]
9	ALCRWGLLL	Her2/neu.5	100	--	278	--	--	2	2/2	2/2		
9	ALBRWGLLV	Her2/neu.5.B3V9	18	33	4.2	285	--	4				
9	HLYQGCQVV	Her2/neu.48	139	307	13	514	1143	3	1/2	0/2	2/2	1/2
9	VLIQRNPQL	Her2/neu.153	23	3909	3.3	1057	--	2				
9	VLIQRNPQV	Her2/neu.153.V9	55	768	135	385	--	3				
9	KIFGSLAFL	Her2/neu.369	36	9.0	19	23	3333	4	6/7	4/7		
9	KLFGSLAFV	Her2/neu.369.L2V9	5.8	7.5	19	17	1270	4				
9	KVFGSLAFV	Her2/neu.369.V2V9	20	19	769	15	29	4				
9	KTFGSLAFV	Her2/neu.369.T2V9	35	13	1010	14	17	4				
10	RILHNGAYSL	Her2/neu 434	278	1000	6667	1276	--	1				
9	ILHNGAYSL	Her2/neu.435	75	358	100	569	--	3	3/3	1/3	2/2	2/2
9	SLISAVVGV	Her2/neu.653.L2V9	7.1	10	16	20	110	5				
9	VVLGVVFGI	Her2/neu.665	14	--	2500	430	2000	2				
9	VLLGVVFGV	Her2/neu.665.L2V9	2.4	19	14	6.0	8000	4				
9	RLLQETELV	Her2/neu.689	21	--	625	34	--	2				
10	VMAGVGSPYV	Her2/neu.773	200	391	13	3700	--	3	1/2	0/2	1/2	1/2
9	CLTSTVQLV	Her2/neu.789	208	457	6.7	308	8000	4	1/4	0/4	1/2	
10	YMIMVKCWMI	Her2/neu.952	20	307	83	116	267	5	0/1	0/1	2/2	2/2
10	YLIMVKCWMV	Her2/neu.952.L2V10	13	56	116	18	84	5				

1. Number of donors yielding a positive response / total tested.
2. Data from ovarian cancer patients.

Table XXVIII. Her2/neu DR supertype primary binding

DR147 Algo Sum	Sequence	Source	DR1 nM	DR4w4 nM	DR7 nM	DR147 Cross-binding
2	LCRWGLLLALLPPGA	Her2/neu.6	53	--	--	1
2	RWGLLLALLPPGAAS	Her2/neu.8	0.42	161	--	2
2	WGLLLALLPPGAAST	Her2/neu.9	0.98	35	--	2
2	GTDMKLRLPASPETH	Her2/neu.28	5000	--	--	0
2	DMKLRLPASPETHLD	Her2/neu.30	5000	--	--	0
2	NLELTYLPTNASLSF	Her2/neu.59	11	118	368	3
3	LTYLPTNASLSFLQD	Her2/neu.62	10	136	78	3
2	TQLFEDNYALAVLDN	Her2/neu.105	94	--	1563	1
2	VCPLHNQEVTAEDGT	Her2/neu.314	--	--	--	0
2	CKKIFGSLAFLPESF	Her2/neu.367	21	--	926	2
2	LSVFQNLQVIRGRIL	Her2/neu.422	28	672	86	3
2	LRELGSGLALIHHNT	Her2/neu.458	161	--	--	1
3	KPDLSYMPIWKFPDE	Her2/neu.605	152	--	8621	1
3	ASPLTSIISAVVGIL	Her2/neu.648	56	--	714	2
2	LTSIISAVVGILLVV	Her2/neu.651	26	--	5102	1
3	VVGILLVVVLGVVFG	Her2/neu.658	--	--	--	0
3	LLVVVLGVVFGILIK	Her2/neu.662	>6250	--	--	0
2	VLGVVFGILIKRRQQ	Her2/neu.666	71	--	781	2
2	ETELVEPLTPSGAMP	Her2/neu.693	833	--	--	1
2	VEPLTPSGAMPNQAQ	Her2/neu.697	>6250	--	--	0
2	ETELRKVKVLGSGAF	Her2/neu.717	313	1286	658	2
2	GENVKIPVAIKVLRE	Her2/neu.743	79	--	807	2
2	IKVLRENTSPKANKE	Her2/neu.752	--	--	--	0
3	KEILDEAYVMAGVGS	Her2/neu.765	--	6164	--	0
3	DEAYVMAGVGSPYVS	Her2/neu.769	100	196	125	3
2	SRLLGICLTSTVQLV	Her2/neu.783	14	375	45	3
2	TVQLVTQLMPYGCLL	Her2/neu.793	22	978	2500	2
3	LLNWCMQIAKGMSYL	Her2/neu.822	6.0	--	208	2
2	ITDFGLARLLDIDET	Her2/neu.861	1042	--	--	0
3	KVPIKWMALESILRR	Her2/neu.883	2.3	652	1316	2
3	PIKWMALESILRRRF	Her2/neu.885	6.3	1286	3205	1
2	IKWMALESILRRRFT	Her2/neu.886	5.3	1125	6250	1
2	GVTVWELMTFGAKPY	Her2/neu.909	3.6	1364	1471	1
3	VWELMTFGAKPYDGI	Her2/neu.912	58	818	676	3
2	GERLPQPPICTIDVY	Her2/neu.938	--	--	--	0
2	QPPICTIDVYMIMVK	Her2/neu.943	75	7500	250	2
2	DVYMIMVKCWMIDSE	Her2/neu.950	179	790	192	3
2	QGFFCPDPAPGAGGM	Her2/neu.1028	--	1957	--	0
3	TDGYVAPLTCSPQPE	Her2/neu.1124	--	--	--	0
2	QPDVRPQPPSPREGP	Her2/neu.1142	7143	--	--	0
2	PSTFKGTPTAENPEY	Her2/neu.1234	--	--	--	0

Table XXIX. DR supertype crossbinding

Sequence	Source	DR1 nM	DR4w4 nM	DR7 nM	DR2w2β1 nM	DR2w2β2 nM	DR6w19 nM	DR5w11 nM	DR8w2 nM	DR147 Binding	Broad Binding (5/8)
RWGLLLALLPPGAAS	Her2/neu.8	0.40	161	--	70	741	--	282	408	2	6
WGLLLALLPPGAAST	Her2/neu.9	1.0	35	--	43	1818	--	80	109	2	5
NLELTYLPTNASLSF	Her2/neu.59	11	118	368	325	2222	2059	4000	2227	3	4
LTYLPTNASLSFLQD	Her2/neu.62	10	136	78	910	357	125	4878	9074	3	6
CKKIFGSLAFLPESF	Her2/neu.367	21	--	926	1300	--	1029	--	--	2	2
LSVFQNLQVIRGRIL	Her2/neu.422	28	672	86	325	270	614	2000	1485	3	6
ASPLTSIISAVVGIL	Her2/neu.648	56	--	714	96	5405	73	--	--	2	4
VLGVVFGILIKRRQQ	Her2/neu.666	71	--	781	827	323	233	43	77	2	7
ETELRKVKVLGSGAF	Her2/neu.717	313	1286	658	4790	3846	2500	3279	1960	2	2
GENVKIPVAIKVLRE	Her2/neu.743	79	--	807	1936	5882	8750	--	--	2	2
DEAYVMAGVGSPYVS	Her2/neu.769	100	196	125	3138	833	1750	7407	860	3	5
SRLLGICLTSTVQLV	Her2/neu.783	14	375	45	414	--	10	1429	--	3	5
TVQLVTQLMPYGCLL	Her2/neu.793	22	978	2500	12	--	1129	--	7101	2	3
LLNWCMQIAKGMSYL	Her2/neu.822	6.0	--	208	1597	17	90	50	120	2	6
KVPIKWMALESILRR	Her2/neu.883	2.3	652	1316	3.4	9.5	1129	2740	6203	2	4
VWELMTFGAKPYDGI	Her2/neu.912	58	818	676	92	200	8750	3704	5506	3	5
QPPICTIDVYMIMVK	Her2/neu.943	75	7500	250	169	7407	2692	4348	9608	2	3
DVYMIMVKCWMIDSE	Her2/neu.950	179	790	192	1936	4762	--	909	1089	3	4

Table XXX. DR3 binding

Sequence	Source	DR3 nM
RLPASPETHLDMLRH	Her2/neu.34	--
SLSFLQDIQEVQGYV	Her2/neu.70	5769
VLIAHNQVRQVPLQR	Her2/neu.84	--
GTQLFEDNYALAVLD	Her2/neu.104	1364
DTILWKDIFHKNNQL	Her2/neu.165	--
ALTLIDTNRSRACHP	Her2/neu.180	8571
KGPLPTDCCHEQCAA	Her2/neu.228	--
LVTYNTDTFESMPNP	Her2/neu.271	--
YNYLSTDVGSCTLVC	Her2/neu.301	--
NQEVTAEDGTQRCEK	Her2/neu.319	--
CYGLGMEHLREVRAV	Her2/neu.342	--
SLAFLPESFDGDPAS	Her2/neu.373	--
PESFDGDPASNTAPL	Her2/neu.378	--
TAPLQPEQLQVFETL	Her2/neu.389	--
LALIHHNTHLCFVHT	Her2/neu.465	968
VHTVPWDQLFRNPHQ	Her2/neu.477	--
WDQLFRNPHQALLHT	Her2/neu.482	333
LQGLPREYVNARHCL	Her2/neu.547	--
VTCFGPEADQCVACA	Her2/neu.574	--
PSGVKPDLSYMPIWK	Her2/neu.601	--
IWKFPDEEGACQPCP	Her2/neu.613	--
HSCVDLDDKGCPAEQ	Her2/neu.632	--
MRRLLQETELVEPLT	Her2/neu.687	--
QMRILKETELRKVKV	Her2/neu.711	938
AIKVLRENTSPKANK	Her2/neu.751	--
NKEILDEAYVMAGVG	Her2/neu.764	--
GMSYLEDVRLVHRDL	Her2/neu.832	1667
VRLVHRDLAARNVLV	Her2/neu.839	882
ARLLDIDETEYHADG	Her2/neu.867	968
ETEYHADGGKVPIKW	Her2/neu.874	--
IKWMALESILRRRFT	Her2/neu.886	682
CWMIDSECRPRFREL	Her2/neu.958	667
FRELVSEFSRMARDP	Her2/neu.969	4225
FSRMARDPQRFVVIQ	Her2/neu.976	1875
FVVIQNEDLGPASPL	Her2/neu.986	--
YRSLLEDDDMGDLVD	Her2/neu.1005	4762
RSLLEDDDMGDLVDA	Her2/neu.1006	--
GDLVDAEEYLVPQQG	Her2/neu.1015	--
QGFFCPDPAPGAGGM	Her2/neu.1028	--
DLTLGLEPSEEEAPR	Her2/neu.1058	--
SDVFDGDLGMGAAKG	Her2/neu.1083	--
LQRYSEDPTVPLPSE	Her2/neu.1109	--
TVPLPSETDGYVAPL	Her2/neu.1117	--
KNGVVKDVFAFGGAV	Her2/neu.1177	--
QGGAAPQPHPPPAFS	Her2/neu.1200	--
DNLYYWDQDPPERGA	Her2/neu.1218	--

TABLE XXXI. HLA Class II Supermotif and Motif-Bearing Epitopes

Sequence	Source	DRB1 *0101 nM	DRB1 *0301 nM	DRB1 *0401 nM	DRB1 *0701 nM	DRB1 *0802 nM	DRB1 *1101 nM	DRB1 *1302 nM	DRB1 *1501 nM	DRB5 *0101 nM	No. of DR Alleles Crossbound
VLGVVFGILIKRRQQ	Her2/neu.666	71	--	--	781	77	43	233	827	323	7
QMRILKETELRKVKV	Her2/neu.711	119	938	>8182	1923	7656	4878	4375	607	34	3
DEAYVMAGVGSPYVS	Her2/neu.769	100	--	196	125	860	7407	1750	3138	833	5
SRLLGICLTSTVQLV	Her2/neu.783	14	--	375	45	--	1429	10	414	--	5
LLNWCMQIAKGMSYL	Her2/neu.822	6.0	--	--	208	120	50	90	1597	17	6
VRLVHRDLAARNVLV	Her2/neu.839	147	882	3058	1087	490	74	81	1422	6061	4
ARLLDIDETEYHADG	Her2/neu.867	--	968	>8182	--	--	--	--	--	--	0
IKWMALESILRRRFT	Her2/neu.886	17	682	3224	4098	731	370	2500	11	2.5	5
VWELMTFGAKPYDGI	Her2/neu.912	58	--	818	676	5506	3704	8750	92	200	5
CWMIDSECRPRFREL	Her2/neu.958	1389	667	>8182	--	--	--	--	--	1333	0

WHAT IS CLAIMED IS

1. An isolated prepared HER2/neu epitope consisting of a sequence selected from the group consisting of the sequences set out in Tables XXIII, XXIV, XXV, XXVI, XXVII, and XXXI.

2. A composition of claim 1, wherein the epitope is admixed or joined to a CTL epitope.

3. A composition of claim 2, wherein the CTL epitope is selected from the group set out in claim 1.

4. A composition of claim 1, wherein the epitope is admixed or joined to an HTL epitope.

5. A composition of claim 4, wherein the HTL epitope is selected from the group set out in claim 1.

6. A composition of claim 4, wherein the HTL epitope is a pan-DR binding molecule.

7. A composition of claim 1, comprising at least three epitopes selected from the group set out in claim 1.

8. A composition of claim 1, further comprising a liposome, wherein the epitope is on or within the liposome.

9. A composition of claim 1, wherein the epitope is joined to a lipid.

10. A composition of claim 1, wherein the epitope is joined to a linker.

11. A composition of claim 1, wherein the epitope is bound to an HLA heavy chain, β2-microglobulin, and strepavidin complex, whereby a tetramer is formed.

12. A composition of claim 1, further comprising an antigen presenting cell, wherein the epitope is on or within the antigen presenting cell.

13. A composition of claim 12, wherein the epitope is bound to an HLA molecule on the antigen presenting cell, whereby when a cytotoxic lymphocyte (CTL) or helper T lymphocyte (HTL) is present that is restricted to the HLA molecule, a receptor on the CTL or HTL binds to a complex of the HLA molecule and the epitope.

14. A clonal cytotoxic T lymphocyte (CTL), wherein the CTL is cultured *in vitro* and binds to a complex of an epitope selected from the group set out in Tables XXIII, XXIV, XXV, XXVI, and XXVII, bound to an HLA molecule.

15. A peptide comprising at least a first and a second epitope, wherein the first epitope is selected from the group consisting of the sequences set out in Tables XXIII, XXIV, XXV, XXVI, XXVII, and XXXI;

wherein the peptide comprise less than 50 contiguous amino acids that have 100% identity with a native peptide sequence.

16. A composition of claim 15, wherein the first and the second epitope are selected from the group of claim 14.

17. A composition of claim 16, further comprising a third epitope selected from the group of claim 15.

18. A composition of claim 15, wherein the peptide is a heteropolymer.

19. A composition of claim 15, wherein the peptide is a homopolymer.

20. A composition of claim 15, wherein the second epitope is a CTL epitope.

21. A composition of claim 20, wherein the CTL epitope is from a tumor associated antigen that is not HER2/neu.

22. A composition of claim 15, wherein the second epitope is a PanDR binding molecule.

23. A composition of claim 1, wherein the first epitope is linked to an a linker sequence.

24. A vaccine composition comprising:

a unit dose of a peptide that comprises less than 50 contiguous amino acids that have 100% identity with a native peptide sequence of HER2/neu, the peptide comprising at least a first epitope selected from the group consisting of the sequences set out in Tables XXIII, XXIV, XXV, XXVI, XXVII, and XXXI; and;

a pharmaceutical excipient.

25. A vaccine composition in accordance with claim 24, further comprising a second epitope.

26. A vaccine composition of claim 24, wherein the second epitope is a PanDR binding molecule.

27. A vaccine composition of claim 24, wherein the pharmaceutical excipient comprises an adjuvant.

28. An isolated nucleic acid encoding a peptide comprising an epitope consisting of a sequence selected from the group consisting of the sequences set out in Tables XXIII, XXIV, XXV, XXVI, XXVII, and XXXI.

29. An isolated nucleic acid encoding a peptide comprising at least a first and a second epitope, wherein the first epitope is selected from the group consisting of the sequences set out in Table XXIII, XXIV, XXV, XXVI, XXVII, and XXXI; and wherein the peptide comprises less than 50 contiguous amino acids that have 100% identity with a native peptide sequence.

30. An isolated nucleic acid of claim 29, wherein the peptide comprises at least two epitopes selected from the sequences set out in claim 29.

31. An isolated nucleic acid of claim 30, wherein the peptide comprises at least three epitopes selected from the sequences set out in claim 29.

32. An isolated nucleic acid of claim 29, wherein the second peptide is a CTL epitope.

33. An isolated nucleic acid of claim 32, wherein the CTL is from a tumor-associated antigen that is not HER2/neu.

34. An isolated nucleic acid of claim 20, wherein the second peptide is an HTL epitope.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/33591

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL : 530/324, 325, 326, 327, 328; 514/12, 13, 14, 15; 536/23.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/324, 325, 326, 327, 328; 514/12, 13, 14, 15; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST 2.0, MEDLINE, BIOSIS, EMBASE search terms: author names, her2, her 2, her2/neu, mhc, peptid?, hla, class I, class II, dr

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KAWASHIMA et al. Identification of HLA-A3 restricted cytotoxic T lymphocyte epitopes from carcinoembryonic antigen and HER-2/neu by primary in vitro immunization with peptide-pulsed dendritic cells. Cancer Research. January 1999, Vol. 59, pages 431-435, 1999, see entire document.	1-34
Y	US 5,876,712 A (CHEEVER et al.) 2 March 1999, see entire document.	1-34
Y	US 5,783,567 A (HEDLEY et al.) 21 July 1998, see entire document.	1-34

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
26 FEBRUARY 2001	10 APR 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ERON SCHWADRON Telephone No. (703) 308-0196

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International application No.
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A. CLASSIFICATION OF SUBJECT MATTER:
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A61K 38/00, 38/38/03, 38/08, 38/10, 38/16; CO7K 7/00, 9/00, 14/435; CO7H 21/02, 21/04